The Journal of Experimental Biology 201, 2815–2820 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1403

TWO TYPES OF mRNA ENCODING MYOSIN REGULATORY LIGHT CHAIN IN CARP FAST SKELETAL MUSCLE DIFFER IN THEIR 3′ NON-CODING REGIONS AND EXPRESSION PATTERNS FOLLOWING TEMPERATURE ACCLIMATION

YASUSHI HIRAYAMA[1], ATSUSHI KOBIYAMA[1], YOSHIHIRO OCHIAI[2] AND SHUGO WATABE[1,*]

[1]*Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan* and [2]*Laboratory of Food Science, Faculty of Education, Ibaraki University, Mito 310-8512, Japan*

*Author for correspondence (e-mail: awatabe@hongo.ecc.u-tokyo.ac.jp)

Accepted 5 August; published on WWW 22 September 1998

Summary

cDNA clones encoding the myosin regulatory light chain (RLC) were isolated from a cDNA library prepared from fast skeletal muscle of the carp *Cyprinus carpio* L. Two types of cDNA clone encoding carp RLC were found with identical deduced amino acid sequences. The two mRNAs differed in the number of polyadenylation signals prior to the poly(A) tail in the 3′ non-coding region. The two mRNA species, with approximate sizes of 1.4 and 0.8 kilobases, were also observed in northern blot analysis. Carp were acclimated for a minimum of 5 weeks to either 10 °C or 30 °C (14 h:10 h light:dark photoperiod). The total levels of mRNA transcripts coding for the RLC and myosin heavy chain were, respectively, 3.3 and 3.9 times higher in cold- than in warm-acclimated fish. Differences in the levels of RLC in mRNA transcripts were largely due to the concentration of the 1.4 kilobase mRNA species.

Key words: carp, *Cyprinus carpio*, myosin regulatory light chain, alternative polyadenylation, temperature acclimation, muscle.

Introduction

Myosin, as well as actin, is a major myofibrillar protein and consists of two heavy chains of approximately 200 kDa and two pairs each of essential (ELC) and regulatory (RLC) light chains of approximately 20 kDa (Harrington and Rodgers, 1984). Myosin is cleaved by limited proteolysis into two functional domains. The head portion, called subfragment-1 (S1), contains ATPase catalytic and actin-binding sites, whereas the tail portion, called the rod, is responsible for thick filament formation. The three-dimensional structure of chicken fast skeletal muscle S1 revealed that two light chains, one each of ELC and RLC, bind noncovalently to a long α-helical chain found at the C-terminal portion of the S1 heavy chain, acting to stabilize the helix (Rayment *et al.* 1993). It is considered that this portion of myosin, called the neck region, acts as a rigid lever arm during the powerstroke of the crossbridge cycle (Uyeda *et al.* 1996; Anson *et al.* 1996).

Eurythermal fish such as carp *Cyprinus carpio* and goldfish *Carassius auratus* show changes in myofibrillar ATPase activity following temperature acclimation (Johnston *et al.* 1975; Heap *et al.* 1985). Maximum force production, contraction speed and Ca^{2+}-sensitivity are altered by temperature acclimation in isolated muscle fibres (Johnston *et al.* 1985). Contraction speed is also modified by the type and ratio of ELCs (Crockford and Johnston, 1990). We reported that the change in myofibrillar ATPase activity was accomplished mainly as a result of the altered expression of myosin isoforms having different ATPase activities (Hwang *et al.* 1990, 1991; Watabe *et al.* 1992, 1994; Guo *et al.* 1994). Recently, we isolated cDNAs encoding three types of carp myosin heavy chain isoforms and showed that the mRNA levels of the three isoforms were dramatically changed following temperature acclimation (Watabe *et al.* 1995; Imai *et al.* 1997; Hirayama and Watabe, 1997). Moreover, we also cloned cDNAs encoding two ELC isoforms, LC1 and LC3, from a carp fast muscle cDNA library and showed that the molar ratio of LC1 mRNA to LC3 mRNA was significantly changed following temperature acclimation (Hirayama *et al.* 1997). Thus, the change in myosin ATPase activity after temperature acclimation seems to be caused by the expression of myosin heavy, and possibly light, chain isoforms.

It is well known that myosin RLC from vertebrate skeletal muscle is quite different from those from vertebrate smooth muscle and non-muscle tissues. The Mg^{2+}-ATPase activity of smooth muscle and non-muscle myosins is regulated by phosphorylation of the RLC. In contrast, contraction of vertebrate skeletal muscle is primarily regulated by binding of Ca^{2+} to the tropomyosin–troponin complex in thin filaments.

Although skeletal myosin RLC of higher vertebrates has also been demonstrated to be phosphorylated (Perrie *et al.* 1973), its physiological significance is still ambiguous, unlike the RLC from smooth muscle and non-muscle tissues. In the case of fish, the skeletal muscle RLC has been purified from a few species and its physicochemical and immunological properties have been well studied (Huriaux and Focant 1977; Watabe *et al.* 1983; Ochiai *et al.* 1988). Moreover, Yancey and Johnston (1982) showed that the fish RLC could also be phosphorylated. However, no information is available on its primary structure; this information would be useful for understanding the physiological roles of the skeletal muscle RLC in the regulation of muscle contraction.

The aim of the present study was to characterize cDNA clones encoding the carp myosin RLC and to examine the coordinated expression of mRNA levels for the myosin heavy and light chains following temperature acclimation.

Materials and methods

Fish

Carp, *Cyprinus carpio* L. (0.5–0.8 kg in body mass), were acclimated to either 10 or 30 °C for a minimum of 5 weeks under a 14 h:10 h light:dark photoperiod (Heap *et al.* 1985). Fast skeletal muscle isolated from the dorsal epaxial muscle was used in all experiments.

Screening of the cDNA library

Fast skeletal muscle cDNA libraries of carp acclimated to 10 and 30 °C (Imai *et al.* 1997; Hirayama *et al.* 1997; Hirayama and Watabe, 1997) were screened using anti-skipjack 5,5′-dithio-bis-2-nitrobenzoic acid light chain (RLC) antiserum (Ochiai *et al.* 1989) using the method of Sambrook *et al.* (1989). XL1-Blue infected with λZAP II cDNA library was cultured on an agar plate at 42 °C for 4 h, and a nitrocellulose filter containing 10 mmol l^{-1} isopropyl-β-*D*-thiogalactopyranoside was then overlaid on the plate and incubated at 37 °C for 4 h. The nitrocellulose filter replica was screened using anti-skipjack RLC antiserum. *In vivo* excision was carried out according to the manufacturer's instructions (Stratagene).

DNA sequence analysis

Various restriction fragments of carp RLC cDNA clones were subcloned into pBluescript II using *Escherichia coli* strain MV1190 as a host bacterium. Sequencing was performed using Dye Deoxy terminator cycle sequence kits with a DNA sequencer (model 373S; Perkin Elmer).

Northern blot analysis

Carp acclimated to 10 and 30 °C were killed, and total RNA was isolated from fast skeletal muscle with a solution containing Isogen, a guanidine-isothiocyanate-based reagent (Nippon Gene) (five fish per acclimation temperature). The concentration of RNA was determined from the absorbance at 260 nm using a Shimadzu UV-160 spectrophotometer. Total RNA (5 μg) was size-fractionated by electrophoresis on a 1.2 % agarose gel in 0.2 mol l^{-1} Mops (pH 7.0) containing 18 % formamide, 0.05 mol l^{-1} sodium acetate and 5 mmol l^{-1} EDTA, and capillary-transferred from the gel to a nylon membrane. pLC2 10-2 (see Fig. 1) was radioactively labelled in the presence of [α-^{32}P]dCTP with a random primer (DNA-labelling kit ver. 2; Takara) and used as a probe for mRNA encoding the regulatory light chain. The oligonucleotide probe 5′-dGGTTGAAGAACTGTTGCAGTTTCTC-3′, which could recognize all three carp myosin heavy chain isoforms (Hirayama and Watabe, 1997), was also synthesized and labelled with [γ-^{32}P]ATP using a Megalabel DNA 5′ end-labelling kit (Takara). This nucleotide sequence region encoding the long α-helix is well conserved among various myosins (Patterson and Spudich, 1996). Hybridization was performed according to Church and Gilbert (1984). The membrane was prehybridized at 65 °C for 14 h in a solution containing 250 mmol l^{-1} Na_2HPO_4 (pH 7.2), 1 mmol l^{-1} EDTA and 7 % SDS. The blot was then hybridized at 65 °C for 18 h in the same solution containing ^{32}P-labelled cDNA probes. After washing with 0.5×SSC (1×SSC is 0.15 mol l^{-1} sodium chloride, 0.015 mol l^{-1} sodium citrate) containing 0.1 % SDS at 65 °C, the blot was exposed to X-ray films for 24 h using an intensifying screen. The hybridized membrane was scanned with a Fujix Bas 1000 computerized densitometer scanner and quantified using the recommended program in the range where there was a linear relationship between the amount of ^{32}P incorporated and the scanned values.

Statistical analyses

A Student's *t*-test was employed to compare differences between values for northern blot analysis between carp acclimated to 10 and 30 °C.

Results and discussion

Cloning of myosin RLC cDNA from carp fast skeletal muscle

When 5×10^4 plaques of fast skeletal muscle cDNA libraries from carp acclimated to either 10 or 30 °C were screened with

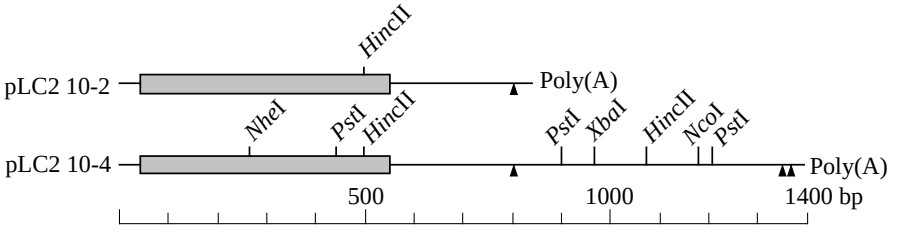

Fig. 1. Partial restriction maps of cDNA clones encoding the two myosin regulatory light chains of carp fast skeletal muscle. Shaded bars represent coding regions and filled triangles indicate the locations of putative polyadenylation signals (AATAAA).

anti-skipjack myosin RLC antiserum, approximately 20 clones were obtained from each of the libraries. Agarose gel electrophoresis together with partial nucleotide sequence analysis revealed that these clones could be separated into two types according to their size. Fig. 1 shows the restriction maps of representative cDNA clones from both types. The two cDNA clones, pLC2 10-2 and pLC2 10-4, had 840 and 1395 nucleotides, respectively, and contained the entire coding region as well as the 3′ non-coding region including poly(A) tails (Fig. 2). Comparison of the nucleotide sequence between the two clones revealed that there were 17 nucleotide differences in corresponding regions. In addition, we detected multiple allelic variants for both shorter and longer mRNAs encoding RLCs in the cDNA library constructed from one carp individual (data not shown). We also found several variations in DNA nucleotide sequences for homologous clones of carp myosin heavy and essential light chains even from one individual (our unpublished data). Although it is difficult to explain the existence of such variations, they may be caused by the tetraploid origin of carp. It has been claimed that duplicate loci appear to remain expressed for millions of years in the case of carp somatotropin (Larhammar and Risinger, 1994).

However, the deduced amino acid sequences of the two clones were identical. Interestingly, pLC2 10-4 had three polyadenylation signals which were arrayed in tandem in the 3′ non-coding region with the poly(A) tail added downstream of the third putative polyadenylation signal. pLC2 10-2 had a polyadenylation signal at the position corresponding to the first site in pLC2 10-4. Therefore, the two cDNAs are probably produced from a single gene by the alternative use of three polyadenylation signals. The other possibility is that the two types of mRNA are transcribed from their respective genes, which have been duplicated during evolution from a single gene. Although most eukaryotic mRNAs possess a single polyadenylation signal, a number of examples of mRNAs with multiple tandem poly(A) sites have been reported (Edwards-Gilbert *et al.* 1997).

```
10-4 CACTTCTTGA GCTTCTTAGA CTTCACACAT ACCGTCTCAG C[ATG]GCACCC AAGAAGGCCA   60
10-2 .......... .......... .......... .......... ..........  ..........  60
                                                    M  A  P  K  K  A     6

10-4 AGAGGAGGGC AGGAGGAGGA GAGGGCTCCT CCAATGTCTT CTCCATGTTT GAGCAGAGCC  120
10-2 .......... .......... .......... .....C..G. ..........  ..........  120
     K  R  R  A  G  G  G  E  G  S  S  N  V  F  S  M  F  E  Q  S     26

10-4 AGATTCAGGA GTACAAAGAG GCTTTCACAA TCATTGACCA GAACAGAGAC GGTATCATCA  180
10-2 Q  I  Q  E  Y  K  E  A  F  T  I  I  D  Q  N  R  D  G  I  I     46

10-4 GCAAAGACGA TCTTAGGGAC GTGTTGGCCT CAATGGGCCA GCTGAACGTG AAGAATGAGG  240
10-2 .....C.... .......... .......... .......... .......... ..........  240
     S  K  D  D  L  R  D  V  L  A  S  M  G  Q  L  N  V  K  N  E     66

10-4 AGCTGGAGGC CATGATCAAG GAAGCTAGCG GCCCAATCAA CTTCACCGTT TTCCTCACCA  300
10-2 .....C.... .......... .......... .......... .......... ..........  300
     E  L  E  A  M  I  K  E  A  S  G  P  I  N  F  T  V  F  L  T     86

10-4 TGTTCGGAGA GAAGCTGAAG GGTGCTGACC CCGAAGACGT CATTGTGTCT GCCTTCAAGG  360
10-2 .......... .......... .......... .......... .......... ..........  360
     M  F  G  E  K  L  K  G  A  D  P  E  D  V  I  V  S  A  F  K    106

10-4 TGCTGGACCC AGAGGGCACC GGCTTCATCA AGAAGCAGTT CCTTGAGGAG CTTCTGACCA  420
10-2 .......... .......... .......... .......A.. .......... ..........  420
     V  L  D  P  E  G  T  G  F  I  K  K  Q  F  L  E  E  L  L  T    126

10-4 CTCAGTGCGA TAGGTTCTCT GCAGAGGAGA TGAAGAATCT GTGGGCCGCC TTCCCCCCAG  480
10-2 .....C.... .......C.. .......... .......... .......... ..........  480
     T  Q  C  D  R  F  S  A  E  E  M  K  N  L  W  A  A  F  P  P    146

10-4 ATGTTGCTGG CAATGTTGAC TACAAGAACA TTTGCTACGT CATCACACAC GGAGAGGAGA  540
10-2 .......... .......... .......... .....C.... .......... ..........  540
     D  V  A  G  N  V  D  Y  K  N  I  C  Y  V  I  T  H  G  E  E    166

10-4 AGGAGGAG[TA] [A]ACGGTCGTG GAGACAAGAC AGAAAATGAA GAGATGAATG TGCATCCCTC  600
10-2 ........ .......... .......... .......... ........C. ..........  600
     K  E  E                                                          166

10-4 ACTGCTTTAC TCTCCCAGTC TGTTCTCTGT CCTCCTTTTC TTACTTTGTG TTCCTTCCTC  660
10-2 .......A.. .......... .......... .......C.. .......... C.T.......  660

10-4 CCTTTCTTCC TTTCCATCAT CTTTGTTACT CTCAAGCACT TACTCTCTCC ATCTCTCCAA  720
10-2 .......... .......... ...C..A... .......... .......... ..........  720

10-4 AGACTTGTCT CGCTGAGGCT GAATTGGGAG GGCGGAGAGG CTCATGACCA CGGTGTCTGT  780
10-2 .......... ...A...... ...C...... ..T....... .......... ..........  780

10-4 CAAGTGGGGA TATGGGATTG TTTTC[AATAA A]ATTAACATC ATTACTGTAT CTCTCACATA  840
10-2 .......... .......... .....[..... .]..........  A AAAAAAAAAA  840

10-4 CTCTCTCTTT CTCTCTGTTT CTCACTCATT ACCCACGACC ACCTCTCATG CATGAACGCT  900
10-4 GCAGCATCTT CTACTGCTGT CTTGAGGGTT GAAGACATCT ATAACTTTTG GCTGAAATCT  960
10-4 CTCTCTAGAT AAATGTGGAA GAGTGCTCAT TCTGAGGGAA AAAGAGGCAA GATGAAGTGA 1020
10-4 TCTTACTCTA TAATAGAGAA ACCAGTCATC ATTTTTGTTT CTTTCGCTTG ACAGTTGACT 1080
10-4 AAAAAGAAAG ACAAAATGAG GGTTTTGTAC CGAGAGAGCT TGGCCTTCTA AAAGTGAGGC 1140
10-4 CAAGCTCATC ACGGAGAGAG TGAGAGAGAC AGACCGTCCA TGGAAGAGAA GAGAATCGGG 1200
10-4 ACTGCAGAGG AAAGAAAACA TATCCTCTTC ACTCCCTCTC TCCCCTCCTC TTTGCTATTT 1260
10-4 CTCTGTCCAT GTTTTTTTTT TTTAAATCTA CCCTTTTGCT TTCTGCATCT GGGCCTGCTT 1320
10-4 TGCTCTGTCA GAAACTCTTC TGTAACC[AAT AAA]AAGACAC AAACTGTG[AA TAAA]ACATCT 1380
10-4 TTTTGTAAAA AAAAA                                                  1395
```

Fig. 2. Nucleotide and deduced amino acid sequences of cDNA clones encoding the two myosin regulatory light chains of carp fast skeletal muscle. Identical nucleotides between the two cDNA clones, pLC2 10-2 (10-2) and pLC2 10-4 (10-4), are shown as periods, and the initiation (ATG) and termination (TAA) codons are shown in bold type. Polyadenylation signals and poly(A) tails are indicated by boxes and underlines, respectively. Refer to the legend of Fig. 1 for further details of the two cDNA clones.

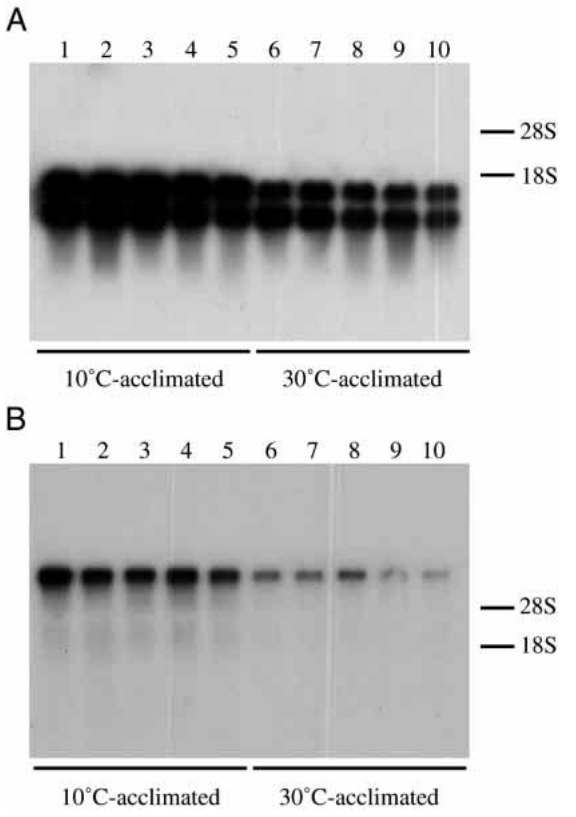

Fig. 3. Northern blot analysis of myosin regulatory light chain (RLC) (A) and heavy chain (B) from carp fast skeletal muscle. Total RNAs prepared from carp acclimated to 10 °C (lanes 1–5) and 30 °C (lanes 6–10) (five fish per acclimation temperature) were size-fractionated in a 1.2 % agarose gel and transferred onto a nylon membrane. The membrane was hybridized with ^{32}P-labelled pLC2 10-2 cDNA clone for the RLC (A) and with an oligonucleotide probe for the myosin heavy chain (B).

Northern blot analysis of carp fast skeletal muscle myosin RLCs

To investigate the mRNA levels of carp RLCs following temperature acclimation, we performed northern blot analyses using the ^{32}P-labelled pLC2 10-2 cDNA clone as a probe. Hybridization patterns with total RNAs from the fast skeletal muscle of carp acclimated to 10 and 30 °C are shown in Fig. 3A. Two bands appeared in all the samples tested. The sizes of the two RNA bands were approximately 1.4 and 0.8 kb. It seems that the two transcripts corresponded to the two types of carp RLC mRNAs described above, since only the slower-migrating band was hybridized with a probe derived from 866–1333 nucleotides of pLC2 10-4 and specific to the longer mRNA (data not shown). Interestingly, the molar ratio of the two transcripts was dependent on the acclimation temperature of the fish. The ratio of the longer to the shorter mRNA was 1.59±0.20 (mean ± S.E.M., N=5) for the 10 °C-acclimated carp and 0.69±0.12 (N=5) for the 30 °C-acclimated fish (P<0.0001). Although it has been reported that different forms of mRNA encoding the same protein have different stabilities and may not be translated with the same efficiency (Edwalds-Gilbert *et al.* 1997), changes in the ratio of the two carp myosin RLC mRNAs could result in altered protein levels. Moreover, the sum of two mRNA signals, based on the content of ribosomal RNA and measured with a Fujix Bas 1000 scanner, was significantly different between the 10 °C- and 30 °C-acclimated carp (P<0.001). Fish acclimated to 10 °C showed approximately 3.3-fold higher mRNA levels than 30 °C-acclimated fish. The level of the longer mRNA in the 10 °C-acclimated carp was 4.2 times higher than in the 30 °C-acclimated carp (P<0.001). However, the concentration of the shorter mRNA was independent of acclimation temperature (P>0.05). These results indicate that changes in the expression of RLC transcripts in the carp following temperature acclimation are primarily regulated by the longer mRNA species.

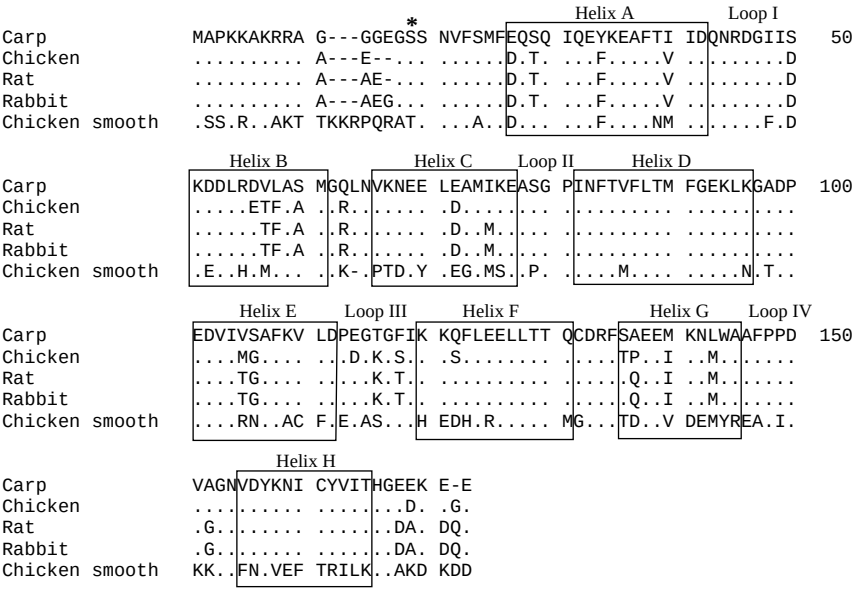

Fig. 4. Comparison of the deduced amino acid sequence of the myosin regulatory light chain (RLC) from carp fast skeletal muscle with those from other vertebrates. Identical and absent amino acid residues in RLCs from chicken (Reinach and Fischman, 1985), rat (Nudel *et al.* 1984) and rabbit fast skeletal muscle (Maeda *et al.* 1990) and from chicken smooth muscle (Messer and Kendrick-Jones, 1988) compared with those in carp RLC are shown by periods and dashes, respectively. The putative myosin light chain kinase phosphorylation site is indicated by an asterisk.

Northern blot analysis of carp fast skeletal muscle myosin heavy chain

We have reported previously that the composition of three types of fast skeletal myosin heavy chain isoform at the mRNA level also changed following temperature acclimation in carp (Imai *et al.* 1997; Hirayama and Watabe, 1997). In the present study, the accumulated levels of mRNAs encoding all three isoforms was 3.9 times higher in the 10 °C-acclimated than in the 30 °C-acclimated carp (P<0.001) (Fig. 3B). Thus, mRNA transcript levels of both the RLC and myosin heavy chain increased to a similar degree with cold-acclimation. The changes in mRNA transcript levels may reflect the altered composition of myosin heavy chain isoforms. To assess the physiological significance of our results, we are now performing additional experiments to obtain data on the time course of cold adaptation, the developmental sequence and the expression pattern in different muscle types.

Comparison of the deduced amino acid sequence of carp fast skeletal muscle RLC with those from other vertebrates

Myosin light chains are members of the EF hand superfamily, which includes calmodulin and troponin-C, consisting of four EF-hand-like domains (Trybus, 1994). Loop I in the first EF hand motif of RLC retains the ability to bind to a metal ion, and this site is probably occupied by Mg^{2+} *in vivo* (daSilva and Reinach, 1991). Fig. 4 compares the amino acid sequence of carp fast skeletal muscle RLC with those from other vertebrate fast and smooth muscles. Carp fast skeletal muscle RLC showed 84.0% sequence identity with those from chicken (Reinach and Fischman, 1985), rat (Nudel *et al.* 1984) and rabbit (Maeda *et al.* 1990) fast skeletal muscle, but only 51.7% identity with that from chicken smooth muscle (Messer and Kendrick-Jones, 1988). It was noted that the amino acid sequences of helices D and H were identical between fast skeletal muscle RLCs. Moreover, the primary structures of loops I, II and IV were well conserved between skeletal muscle RLCs. In addition, it is known that the skeletal muscle RLC of higher vertebrates is phosphorylated by skeletal muscle myosin light chain kinase both *in vivo* and *in vitro* at Ser-15 (Perrie *et al.* 1973; Sweeney and Stull, 1990). This serine residue is also conserved in carp skeletal muscle RLC (Fig. 4). Although the phosphorylation site has not yet been identified, fish RLC is also phosphorylated both *in vivo* and *in vitro* (Yancey and Johnston, 1982). These observations suggest that carp RLC may also be phosphorylatable by skeletal muscle myosin light chain kinase at this serine residue.

S.W. was supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, and from the Asahi Glass Foundation, Japan. Y.H. was supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

References

ANSON, M., GEEVES, M. A., KURZAWA, S. E. AND MANSTEIN, G. (1996). Myosin motors with artificial lever arms. *EMBO J.* **15**, 6069–6074.

CHURCH, G. M. AND GILBERT, W. (1984). Genomic sequencing. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1991–1995.

CROCKFORD, T. AND JOHNSTON, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of the common carp (*Cyprinus carpio* L.). *J. comp. Physiol.* B **160**, 23–30.

DASILVA, A. C. R. AND REINACH, F. C. (1991). Calcium binding induces conformational changes in muscle regulatory proteins. *Trends biochem. Sci*. **16**, 53–57.

EDWALDS-GILBERT, G., KRISTEN, L. V. AND MILCAREK, C. (1997). Alternative poly(A) site selection in complex transcription units: means to an end? *Nucleic Acids Res*. **25**, 2547–2561.

GUO, X.-F., NAKAYA, M. AND WATABE, S. (1994). Myosin subfragment-1 isoforms having different heavy chain structures from fast skeletal muscle of thermally acclimated carp. *J. Biochem.* **116**, 728–735.

HARRINGTON, W. F. AND RODGERS, M. E. (1984). Myosin. *A. Rev. Biochem*. **53**, 35–73.

HEAP, S. P., WATT, P. W. AND GOLDSPINK, G. (1985). Consequences of thermal change on the myofibrillar ATPase of five freshwater teleosts. *J. Fish Biol*. **26**, 733–738.

HIRAYAMA, Y., KANOH, S., NAKAYA, M. AND WATABE, S. (1997). The two essential light chains of carp fast skeletal myosin, LC1 and LC3, are encoded by distinct genes and change their molar ratio following temperature acclimation. *J. exp. Biol*. **200**, 693–701.

HIRAYAMA, Y. AND WATABE, S. (1997). Structural differences in the cross-bridge head of temperature-associated myosin subfragment-1 isoforms from carp fast skeletal muscle. *Eur. J. Biochem*. **246**, 380–387.

HURIAUX, F. AND FOCANT, B. (1977). Isolation and characterization of the three light chains from carp white muscle myosin. *Archs. int. Physiol. Biochim.* **85**, 917–929.

HWANG, G.-C., OCHIAI, Y., WATABE, S. AND HASHIMOTO, K. (1991). Changes of carp myosin subfragment-1 induced by temperature acclimation. *J. comp. Physiol.* B **161**, 141–146.

HWANG, G.-C., WATABE, S. AND HASHIMOTO, K. (1990). Changes in carp myosin ATPase induced by temperature acclimation. *J. comp. Physiol.* B **160**, 233–239.

IMAI, J., HIRAYAMA, Y., KIKUCHI, K., KAKINUMA, M. AND WATABE, S. (1997). cDNA cloning of myosin heavy chain isoforms from carp fast skeletal muscle and their gene expression associated with temperature acclimation. *J. exp. Biol*. **200**, 27–34.

JOHNSTON, I. A., DAVISON, W. AND GOLDSPINK, G. (1975). Adaptation in Mg^{2+}-activated myofibrillar ATPase activity induced by temperature acclimation. *FEBS Lett.* **50**, 293–295.

JOHNSTON, I. A., SIDELL, B. D. AND DRIEDZIC, W. R. (1985). Force–velocity characterization and metabolism of carp muscle fibres following temperature acclimation. *J. exp. Biol.* **119**, 239–249.

LARHAMMAR, D. AND RISINGER, C. (1994). Molecular genetic aspects of tetraploidy in the common carp *Cyprinus carpio*. *Molec. phylogenet. Evol.* **3**, 59–68.

MAEDA, K., MÜLLER-GERHARDT, E. AND WITTINGHOFER, A. (1990). Sequence of two isoforms of myosin light chain 2 isolated from a rabbit fast skeletal muscle lambda library. *Nucleic Acids Res*. **18**, 6687.

MESSER, N. G. AND KENDRICK-JONES, J. (1988). Molecular cloning and sequencing of the chicken smooth muscle myosin regulatory light chain. *FEBS Lett*. **234**, 49–52.

NUDEL, U., CALVO, J. M., SHANI, M. AND LEVY, Z. (1984). The

nucleotide sequence of a rat myosin light chain 2 gene. *Nucleic Acids Res*. **12**, 7175–7186.

OCHIAI, Y., KOBAYASHI, T., HANDA, A., WATABE, S. AND HASHIMOTO, K. (1989). Immunological cross-reactivity of myosin light chains from various species of fish. *Nippon Suisan Gakkaishi* **55**, 2151–2156.

OCHIAI, Y., WATABE, S. AND HASHIMOTO, K. (1988). Physicochemical and immunological properties of myosin light chains from the ordinary muscle of marine teleost fishes. *Comp. Biochem. Physiol*. **90**B, 347–353.

PATTERSON, B. AND SPUDICH, J. A. (1996). Cold-sensitive mutations of *Dictyostelium* myosin heavy chain highlight functional domains of the myosin motor. *Genetics* **143**, 801–810.

PERRIE, W. T., SMILLIE, L. B. AND PERRY, S. J. (1973). A phosphorylated light-chain component of myosin from skeletal muscle. *Biochem. J*. **135**, 151–164.

RAYMENT, I., RYPNIEWSKI, W. R., SCHMIDT-BÄSE, K., SMITH, R., TOMCHICK, D. R., BENNING, M. M., WINKELMANN, D. A., WESENBERG, G. AND HOLDEN, H. M. (1993). Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* **261**, 50–58.

REINACH, F. C. AND FISCHMAN, D. A. (1985). Recombinant DNA approach for defining the primary structure of monoclonal antibody epitopes. The analysis of a conformation-specific antibody to myosin light chain 2. *J. molec. Biol*. **181**, 411–422.

SAMBROOK, J., FRITSCH, E. F. AND MANIATIS, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

SWEENEY, H. L. AND STULL, J. T. (1990). Alteration of cross-bridge kinetics by myosin light chain phosphorylation in rabbit skeletal muscle: implications for regulation of actin–myosin interaction. *Proc. natn. Acad. Sci. U.S.A.* **87**, 414–418.

TRYBUS, K. M. (1994). Role of myosin light chains. *J. Muscle Res. Cell Motil*. **15**, 587–594.

UYEDA, T. Q. P., ABRAMSON, P. D. AND SPUDICH, J. A. (1996). The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc. natn. Acad. Sci. U.S.A.* **93**, 4459–4464.

WATABE, S., DINH, T. N.-L., OCHIAI, Y. AND HASHIMOTO, K. (1983). Immunochemical specificity of myosin light chains from mackerel ordinary and dark muscles. *J. Biochem*. **94**, 1409–1419.

WATABE, S., GUO, X.-F. AND HWANG, G.-C. (1994). Carp express specific isoforms of the myosin cross-bridge head, subfragment-1, in association with cold and warm temperature acclimation. *J. therm. Biol*. **4**, 261–268.

WATABE, S., HWANG, G.-C., NAKAYA, M., GUO, X.-F. AND OKAMOTO, Y. (1992). Fast skeletal myosin isoforms in thermally acclimated carp. *J. Biochem*. **111**, 113–122.

WATABE, S., IMAI, J., NAKAYA, M., HIRAYAMA, Y., OKAMOTO, Y., MASAKI, H., UOZUMI, T., HIRONO, I. AND AOKI, Y. (1995). Temperature acclimation induces light meromyosin isoforms with different primary structures in carp fast skeletal muscle. *Biochem. biophys. Res. Commun.* **208**, 118–125.

YANCEY, P. H. AND JOHNSTON, I. A. (1982). Effect of electrical stimulation and exercise on the phosphorylation state of myosin light chains from fish skeletal muscle. *Pflügers Arch.* **393**, 334–339.