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# 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

YASUSHI HIRAYAMA[1], SATOSHI KANOH[2], MISAKO NAKAYA[1] 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, Japan* and [2]*Laboratory of Marine Biopolymer Chemistry, Department of Chemistry of Fishery Resources, Mie University, Tsu, Mie 514, Japan*

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**Summary**

**cDNA libraries were constructed from fast skeletal muscles of carp acclimated to 10 and 30 °C for a minimum of 5 weeks and were screened for myosin alkali light chains, LC1 and LC3, using an anti-skipjack LC1 polyclonal antibody. Two types of LC1 cDNA clone were isolated and termed LC1a and LC1b: their nucleotide sequences showed 92 % homology. The ratio of LC1a to LC1b cDNA clones isolated was approximately 3:1, showing no apparent changes following temperature acclimation. The occurrence of the two isoforms was further confirmed by N-terminal amino acid sequencing of purified LC1. No isoform was, however, detected for LC3, while homology in the overlapping region between LC1a and LC3 cDNAs was only 65 % even after the most probable alignment. Southern blot analyses probed with cDNA clones specific to LC1a and LC3 showed different hybridization patterns from each other, demonstrating that carp LC1 and LC3 are encoded by different genes. These results are in marked contrast to those from higher vertebrates which express LC1 and LC3 from a single gene by alternative RNA transcription and two modes of splicing. Northern blot analysis showed that the ratios of LC3/LC1 mRNAs were significantly higher (3.93) in 30 °C-acclimated than in 10 °C-acclimated (3.10) carp.**

Key words: carp, fast skeletal muscle, myosin light chain, cDNA, temperature acclimation, *Cyprinus carpio*.

## Introduction

Myosin is a major protein component in the contractile apparatus, consisting of two heavy chains of approximate molecular mass 200 kDa and four light chains of approximate molecular mass 20 kDa. The four myosin light chains are classified into two distinct functional types: two essential light chains released from heavy chains by alkaline treatment and two regulatory light chains dissociated with 5,5′-dithio-bis-2-nitrobenzoic acid (Harrington and Rodgers, 1984). Although the functions of fast skeletal myosin light chains are not fully understood, their three-dimensional structures, together with that of myosin subfragment-1 (S1) heavy chain, have recently been elucidated for chicken (Rayment *et al.* 1993). Myosin light chains surround the long α-helix in the 20 kDa tryptic fragment of S1 heavy chain and stabilize the neck region extending to myosin subfragment-2.

Two alkali light chains from vertebrate fast skeletal muscle myosin are also called LC1 and LC3 and consist of approximately 190 and 150 amino acid residues, respectively (Weeds and Lowey, 1971). Amino acid sequence analysis revealed that LC1 and LC3 from rabbit (Frank and Weeds, 1974) and chicken (Matsuda *et al.* 1981*a*,*b*) have an identical C-terminal sequence composed of 141 amino acid residues, but differ from each other in the length and the sequence of their N-terminal portions. cDNA clones of chicken LC1 and LC3 also show identical sequences in the region encoding the C-terminal 141 amino acid residues, as well as in the 3′ non-coding region (Nabeshima *et al.* 1982). Furthermore, genomic DNA sequence analyses of LC1 and LC3 from chicken (Nabeshima *et al.* 1984), mouse (Robert *et al.* 1984) and rat (Periasamy *et al.* 1984) indicate that they are encoded by a single gene and produced by alternative transcription and two modes of splicing.

As is the case for other vertebrates, fish fast skeletal muscle myosins contain two alkali light chains, LC1 and LC3 (Huriaux and Focant, 1977, 1985; Watabe *et al.* 1982; Ochiai *et al.* 1988, 1990; Rowlerson *et al.* 1985). Although LC1 and LC3 have been isolated from fast skeletal muscles of fish, including carp (Huriaux and Focant, 1977; Ochiai *et al.* 1988), their amino acid sequences are known only for mullet (Dalla Libera *et al.* 1991). Surprisingly, differences in the

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

amino acid sequence between mullet LC1 and LC3 are found in all the regions, including the C-terminal portions, in marked contrast to avian and mammalian myosin light chains. Dalla Libera *et al.* (1991) claimed that the two myosin alkali light chains of fish fast skeletal muscle originate from two different genes.

In this paper, we describe the isolation of cDNA clones encoding fast skeletal muscle myosin LC1 and LC3 from carp acclimated to 10 and 30 °C. cDNA clones encoding two light chains were used to demonstrate the mode of their gene expression as well as possible changes in association with 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 photoperiodic regime (Heap *et al.* 1985), and dorsal fast skeletal muscles were used for the following experiments. The hepatopancreatic tissue of carp was freshly isolated and used for Southern blot analysis.

### *Preparation of myosin alkali light chains*

Carp myosin was prepared from the dorsal skeletal muscle using the procedure for requiem shark myosin (Kanoh *et al.* 1983). Dissociation and purification of myosin light chains were performed according to the method of Lowey and Holt (1972) with some modifications as follows. Myosin was dialyzed at 4 °C overnight against 10 mmol $l^{-1}$ Tris–HCl (pH 7.5) containing 4 mol $l^{-1}$ urea, 15 mmol $l^{-1}$ KCl and 10 mmol $l^{-1}$ 2-mercaptoethanol. Myosin heavy chains were precipitated with 10 volumes of ice-cold water and removed by centrifugation. The resulting supernatant was applied to a DEAE-Toyopearl 650M column (3 cm×42 cm) equilibrated with the same buffer. Myosin light chains were eluted with a linear gradient up to 0.2 mol $l^{-1}$ KCl.

### *SDS–PAGE*

SDS–PAGE was carried out using the method of Laemmli (1970), on 7.5 % to 20 % polyacrylamide gradient slab gels containing 0.1 % SDS. Molecular mass markers were ovalbumin (45 kDa), carbonic anhydrase (29 kDa) and α-lactalbumin (14 kDa). Gels were stained with Coomassie Brilliant Blue R-250 and destained with a solution containing 25 % methanol and 7 % glacial acetic acid.

### *Protein sequence analysis*

Carp myosin alkali light chains were digested at 25 °C for 3 min in 25 mmol $l^{-1}$ Tris–HCl (pH 7.5) containing 50 mmol $l^{-1}$ KCl using trypsin at an enzyme-to-protein mass ratio of 1:40. The tryptic digests were size-fractionated by SDS–PAGE, and peptide bands were electroblotted to a polyvinylidene difluoride membrane, cut into sheets and applied to a protein sequencer model 476A (Perkin Elmer Applied Biosystems) essentially according to the method of Matsudaira (1987).

### *Construction of cDNA library*

Total RNA was prepared from the dorsal skeletal muscle of carp acclimated to 10 or 30 °C, as described by Chomzynski and Sacchi (1987). Poly $(A)^+$ mRNA was purified using an mRNA purification kit (Pharmacia). Carp muscle cDNA was synthesized using a kit (Pharmacia) and 5 μg of mRNA with an oligo-dT primer. The cDNA product was inserted into λZAP II phage vector according to the manufacturer's instructions (Stratagene).

### *cDNA cloning*

Carp skeletal muscle cDNA library was screened using anti-skipjack LC1 polyclonal antibody (Ochiai *et al.* 1989*b*) by the method of Sambrook *et al.* (1989). *Escherichia coli* (XL1-Blue) infected with the 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 overlaid to the plate and incubated at 37 °C for 4 h. The nitrocellulose filter replica was screened with anti-skipjack polyclonal antibody. *In vivo* excision was carried out according to the manufacturer's instructions (Stratagene).

### *DNA sequence analysis*

cDNA restriction fragments of carp myosin alkali light chains were subcloned into plasmid vector pBluescript II. Sequencing was performed using a DNA sequencer model 373A (Perkin Elmer Applied Biosystems) after labelling DNA with Dye Deoxy terminator cycle sequence kits.

### *Southern and northern blot analyses*

For Southern blot analysis, genomic DNA was prepared from freshly killed carp hepatopancreas by the method of Ausubel *et al.* (1987). Carp genomic DNA (15 μg) was digested with restriction enzymes including *Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI and *Xho*I. The digests were size-fractionated by electrophoresis in a 0.7 % agarose gel and transferred to a nylon membrane. The membrane was prehybridized at 42 °C for 14 h in a solution containing 5×SSC (1×SSC is 0.15 mol $l^{-1}$ sodium chloride, 0.015 mol $l^{-1}$ sodium citrate), 10 % dextran sulphate, 5×Denhardt's solution (1×Denhardt's is 0.1 % Ficoll 400, 0.1 % polyvinylpyrrolidone, 0.1 % bovine serum albumin), 0.5 % SDS, 50 % formamide and 100 μg $ml^{-1}$ of heat-denatured calf thymus DNA. The blot was then hybridized at 42 °C for 18 h in the same solution containing $^{32}$P-labelled cDNA probes which had been randomly primed in the presence of [$^{32}$P]dCTP. After washing with 1×SSC containing 0.1 % SDS at 65 °C, the blot was exposed to X-ray films for 24 h using an intensifying screen.

For northern blot analysis, total RNA was isolated from freshly killed carp fast skeletal muscle with an Isogen solution (Nippon Gene). RNA was size-fractionated by electrophoresis in a 0.9 % agarose gel containing 18 % formamide. Subsequent procedures were the same as in the case of Southern blot analysis, except that dextran sulphate was removed from the hybridizing solution. The hybridized membrane was scanned with a Fujix Bas 1000 computerized densitometer scanner and

quantified using a recommended scanning program. cDNA encoding carp skeletal α-actin (Watabe *et al.* 1995*a*) was also used for northern blot analysis as reference.

### *Statistical analysis*

Student's *t*-test was employed to compare differences in LC3/LC1 mRNA ratios between carp acclimated to 10 and 30 °C. $P<0.05$ was accepted as the fiducial limit of significance.

## Results

### *Nucleotide sequences of carp myosin alkali light chain cDNAs*

For each cDNA library, $5\times10^4$ plaques were screened using anti-skipjack LC1 antibody: 10–12 clones showed a positive reaction for both libraries. Partial nucleotide sequence analysis of these clones revealed the presence of two types of LC1, which were thereafter named LC1a and LC1b. At each acclimation temperature, LC1a was the predominant clone (seven out of nine in the 10 °C-acclimated fish and five out of seven in the 30 °C-acclimated fish). Three clones corresponding to LC3 cDNA were isolated from each library and they were found to have an identical nucleotide sequence.

Fig. 1 shows the restriction maps of cDNA clones encoding LC1a, LC1b and LC3. LC1a and LC3 cDNA clones, represented by pLC1a30-1 and pLC3 30-1, respectively, contained the entire coding and non-coding regions, whereas the longest LC1b cDNA clones, pLC1b10-1 and pLC1b10-2, were not full length, lacking a polyadenylation signal and a 5′ non-coding region, respectively. However, pLC1b10-1 and pLC1b10-2 had an identical nucleotide sequence in their overlapping region, demonstrating that they were probably products from the same transcript. The nucleotide sequences of LC1a, LC1b and LC3 cDNAs have been registered to the DDBJ/EMBL/GenBank databases under the accession numbers D85139, D85140 and D85141, respectively.

Widespread differences were found in the nucleotide sequence of LC1a and LC1b cDNA clones, including coding and non-coding regions. Deletions and insertions were found even in the 5′ and 3′ non-coding regions (Fig. 2), resulting in 92 % identity for their overlapping sequence. When the nucleotide sequences of LC1a and LC3 cDNA clones were compared, substitutions were observed in all the regions with much higher frequencies than for LC1a and LC1b (Fig. 3). It has been reported for higher vertebrates that LC1 and LC3 mRNAs share an identical nucleotide sequence in their 3′ region encoding the C-terminal 141 amino acid residues, as well as in their 3′ non-coding region (indicated by the large box in Fig. 3). However, there were many variations in the coding and 3′ non-coding regions between carp LC1a and LC3 cDNAs, indicating that carp LC1 and LC3 are encoded by different genes.

### *Deduced amino acid sequences of carp myosin alkali light chains*

The amino acid sequences of the two types of LC1 (LC1a and LC1b) and LC3 were deduced from cDNA nucleotide sequences, predicting 193 and 151 amino acid residues, respectively (Fig. 4). The two types of carp LC1 contained the so-called difference peptide in the N-terminal regions, where Ala, Pro and Lys are abundant: a similar result has been obtained for other vertebrate LC1s, such as those from rabbit (Frank and Weeds, 1974) and chicken (Matsuda *et al.* 1981*a*,*b*). There were four amino acid substitutions throughout

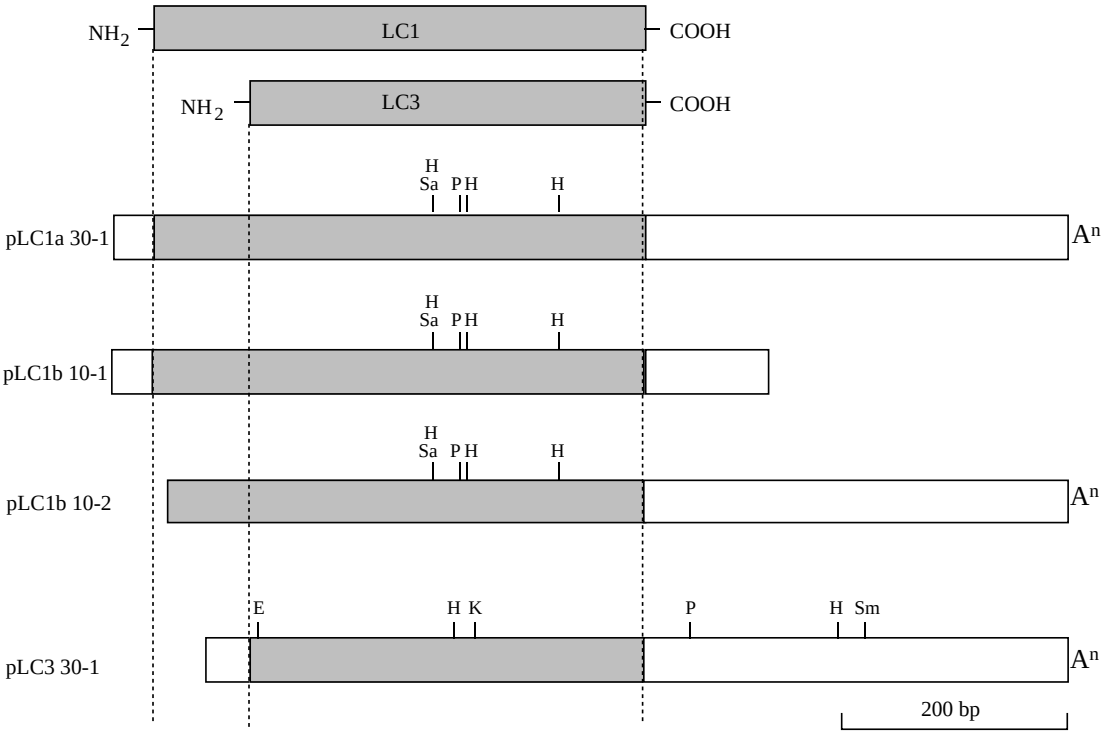


Fig. 1. Partial restriction maps of cDNA clones encoding carp fast skeletal myosin alkali light chains. pLC1a30-1 and pLC3 30-1 were isolated from the 30 °C-acclimated carp cDNA library, while pLC1b10-1 and pLC1b10-2 were isolated from the 10 °C-acclimated carp. Open bars indicate 5′ and 3′ non-coding regions, while shaded bars indicate coding regions. Restriction enzymes: H, *Hin*cII; Sa, *Sal*I; P, *Pst*I; E, *Eco*RI; K, *Kpn*I; Sm, *Sma*I. LC1 and LC3 denote myosin alkali light chains with higher and lower molecular masses, respectively. $A^n$, poly A tails.

```
LC1a         CCTGTTTTGACTTCCCTCCCGGCGGACAAGTAGTC-------------TAAAAAATGGCACCAAAGAAGGACGCTAAGAAGCCTGAGCCTGCCA   81
LC1b    CCACTC.....G.........T............A...A.AAAAAAAATAAAA.....|...|.....T...................C......C...  100

LC1a    AGAAAGCAGAGCCTGCACCTGCCCCCGCTCCCGCGCCCGCACCCGAGGCCCCACCTAAACCCGCAGCAGTAGATCTGTCCGGTGTGAAGGTCGATTTCAA  181
LC1b    .............C.............G........T..C.....................................A...........T........  200

LC1a    CCAGGACCAGCTGGAAGATTACAGGGAGGCCTTCGGACTTTTCGACAGAGTTGGTGACAACAAAGTAGCCTACAACCAGATTGCAGATATCATGCGTGCA  281
LC1b    ..........T..........T.....................................G.....................................  300

LC1a    CTGGGACAGAACCCAACCAACAAAGAGGTTACAAAGATCCTGGGCAACCCTACCGCTCATGAAATGGCCAACAAGAGAGTCGACTTTGAGGGTTTCCTGC  381
LC1b    ..................................................T.............A.............................  400

LC1a    CCATGCTGCAGTTTGTCGTCAACAGCCCAAACAAGGCAACATACGAGGACTATGTTGAGGGTCTGCGTGTATTCGATAAGGAGGGCAACGGAACAGTAAT  481
LC1b    ..............G.............................T.....................................A...........T..  500

LC1a    GGGTGCTGAGCTGCGTATCGTCTTGTCAACACTGGGTGAGAAAATGACTGAAGTTGAGATCGATGCTCTCATGCAAGGCCAGGAGGATGAAAATGGCTGT  581
LC1b    ......C...T.....................................A.....C..............T.............................  600

LC1a    GTGAACTATGAGGCTTTCGTCAAACACATCATGTCTGTG|TAA|GAAGTCGGAGTTGTGAGGAAACTGAAGCGTTTCTCCAGATTCCATGATGTCAGGACAT  681
LC1b    ..........................................|...|.......C.............................................  700

LC1a    CCACACAATGTTTCCAAA-CCAACTCAGAAATGGATAAAAGGGACACGGGATGTTAGTCATAAACAATTATTTTGTTATTTAAGTT--------TCCTAT  772
LC1b    .......G.A........A.................G..........A.-.....................--------.....-...ATTGTGTG......  790

LC1a    TTTTT--CCACTTGAC-------TCTTTTGTCCATTCTTGGATGCCATTCATATTTTTTCCAGCTAATTTTCTTGGTGTTGTTCGTGCCGGGACAAGCCT  863
LC1b    .....TT.........TCCTATT.........T.........A......G.....................................................  890

LC1a    CTCCGTAGCGGAACGGTTTTGAGGGGTGGGGGTGTAGGGGGGCATCATAGTTACACAAAAATAAAAACCACCCC--TTGACCCCTCACTGCCTGGATCAC  961
LC1b    .........A...T....G.........T...G---..........G...C............T..........CC..............T.........  987

LC1a    AAAGTCAAAGGTGCTAAAAATACTGCCATTAATAGACTGGCTGCAAAACCTCCTTCCCCTTTCCCCAGAGTCTTAATTTATTTTTTGCCTCTGTCAATTC 1061
LC1b    ..................................................T.....................................................  1087

LC1a    TCA|TAATAAA|CTTTTCACAAAGTAAAAAAAAAA                                                              1093
LC1b    ....|......|.....--.....G.........AAAAAAAA                                                        1125
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Fig. 2. Nucleotide sequences of cDNAs encoding two types of carp myosin alkali light chain 1. LC1a and LC1b are the major and minor component, respectively. Identical and missing nucleotides between the two types of LC1 are shown by periods and dashes, respectively. The ATG initiation codon, the TAA termination codon and the AATAAA polyadenylation signal are boxed; the poly A tails are underlined.

```
LC1a    CCTGTTTTGACTTCCCTCCCGGCGGACAAGTAGTCTAAAAA|ATG|GCACCAAAGAAGGACGCTAAGAAGCCTGAGCCTGCCAAGAAAGCAGAGCCTGCACC  100
LC3

LC1a    TGCCCCCGCTCCCGCGCCCGCACCCGAGGCCCCACCTAAACCCGCAGCAGTAGATCTGTCCGGTGTGAAGGTCGATTTCAACCAGGACCAGCTGGAAGAT  200
LC3                  CCGGCTTCTTGACTTCTGCCCCTCAGCTCCTGCACCCAAAAACTCCATCA..GCT.GA..A...TCTGCT.....GA.T..G..C   85

LC1a    TACAGGGAGGCCTTCGGACTTTTCGACAGAGTTGGTGACAACAAAGTAGCCTACAACCAGATTGCAGATATCATGCGTGCACTGGGACAGAACCCAACCA  300
LC3     .T..AA........T..T..C.....................G..T............G.C..T..C..........C.............C....  185

LC1a    ACAAAGAGGTTACAAAGATCCTGGGCAACCCTACCGCTCATGAAATGGCCAACAAGAGAGTCGACTTTGAGGGTTTCCTGCCCATGCTGCAGTTTGTCGT  400
LC3     ....G..C..G.AG..A........TG....AT.T.....C..T...........A...A.T........T.C.........A......A..ACC---..  282

LC1a    CAACAGCCCAAACAAGGCAACATACGAGGACTATGTTGAGGGTCTGCGTGTATTCGATAAGGAGGGCAACGGAACAGTAATGGGTGCTGAGCTGCGTATC  500
LC3     TG..GCTGTCC.G....GT..C..T..T.....C..............C..C.....C..A...........C.....G.....C..............T  382

LC1a    GTCTTGTCAACACTGGGTGAGAAAATGACTGAAGTTGAGATCGATGCTCTCATGCAAGGCCAGGAGGATGAAAATGGCTGTGTGAACTATGAGGCTTTCG  600
LC3     ..GC.C.................G.....C..GCCC..A..T..CT.........G..A...........G..C...A....CC.........A.....  482

LC1a    TCAAACACATCATGTCTGTG|TAA|GAA---GTC-G------GAGTTGTGAGGAAA-CTGAAGCGTTTCTCCAGATTCCATGATGTCAGGACATCCACACAA  689
LC3     ....G..........C...|...|...ACC...C.CTGAGA....G....A...GG.....CTA.A...G....CC.....G.............TT.--  580

LC1a    TGTTTCCAA-ACCAACTCAGAAATGGATAAAAGGGAC-ACGGGATGTTAGTCATAAACAATT-ATTTTG------TTATTTAAGTTT-CCTATTTTTTCC  779
LC3     .....TG..GACCAA-T---.........-.....A....T.T.......C.C.TC.....C...CAG...CCCCCCT......GTT..CT..CC.C.CGC..  672

LC1a    ACTTGACTCTTTTGTCCATTCTT---GGATGCCATTCA-TATTTTT-TCCAGCTAATTTTCTTGG---TGTTGTTCGTGCCGGGACAAGCCTCTCCGTAG  871
LC3     ....TTT.T....T.TTT..T..TTG...A.T..CA.CG.CC...CA.G...T.G.CCC..C.C.CCT.C......CGC.....G.G-...G-...AC..  770

LC1a    CGGAACGGTTTTGAGGGGTGGGGGTGTAGG---GGGGCA-TCAT--AGTTACACAAAAATAAAAACCACCCCTTGACCCCTCACTGCCTGGATCACAA-A  964
LC3     T..G.-----.G...AC-.....AA.A.A.AAAA..C..C....GA...G.GGGTTGGT.T--------.TT...T-.T..A.C.AT..A..G.T....C.  857

LC1a    GTCAA----AG----GTGCTAAAAATAC-TGCCATTAATA--GACTGGCTGCAAAACCTCCTTCCCCTTTCCCCAGAGTCTTAATTTATTTTTTGCCTCT 1053
LC3     .-..CCGCT..CTGT...T....GGC..A..A....GC.GTG.C.A......C.-----.T-......CA....----------.....-------.CA.A.A  936

LC1a    GTCAATTCTCAT|AATAAA|CTTTTCACAAAGTAAAAAAAAA                                                       1093
LC3     ....-..-...C|......|.....TT.C----AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA    1006
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Fig. 3. Nucleotide sequences of cDNA clones encoding carp myosin alkali light chains. Identical and missing nucleotides are shown by periods and dashes, respectively, in comparing the predominating LC1 (LC1a) with LC3. The large box indicates where LC1 and LC3 from avian and mammalian species share an identical nucleotide sequence. Refer to the legend of Fig. 2 for an explanation of the smaller boxes and underlining.

```
LC1a  MAPKKDAKKP EPAKKAEPAP APAPAPAPEA PPKPAAVDLS GVKVDFNQDQ   50
LC1b  .......... ..P..... .......... ....------ ----------   50
LC3                                                MAGE.SA..    9

LC1a  LEDYREAFGL FDRVGDNKVA YNQIADIMRA LGQNPTNKEV TKILGNPTAD  100
LC1b  .......... ......S... .......... .......... ..--------  100
LC3   I..FK..... .......... ...V...... ........D. K...D.S..   59

LC1a  EMANKRVDFE GFLPMLQFVV NSPNKATYED YVEGLRVFDK EGNGTVMGAE  150
LC1b  ---------- ---------- ---------- ---------- ----------  150
LC3   D.....I..D A.....KT-. DAVQ.G..D. ----------.. ----------  108

LC1a  LRIVLSTLGE KMTEVEIDAL MQGQEDENGC VNYEAFVKHI MSV         193
LC1b  .......... ..N.A..... .......... .......... ...       193
LC3   ..-------- ....P...S. ..--------S .H..D..... ...       151
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Fig. 4. Comparison of deduced amino acid sequences of carp myosin alkali light chains. Identical and missing amino acids compared with those in LC1a are shown by periods and dashes, respectively, in LC1b and LC3. Underlined sequences were confirmed by N-terminal amino acid sequence analysis as shown in Fig. 5. A box indicates the area where LC1 and LC3 share an identical amino acid sequence in avian and mammalian species.

the sequence of LC1a and LC1b, resulting in a 98 % sequence homology.

Comparison of the amino acid sequence of the predominant LC1a isoform with that of LC3 in their overlapping region, after the most probable alignment, revealed 31 amino acid substitutions (Fig. 4) with 84 % sequence homology. LC3 lacked one residue between residues 76 and 84 from the N terminus; in Fig. 4, this gap was tentatively inserted between residues 77 and 78. One amino acid deletion in LC3 has also been reported for mullet (Dalla Libera *et al.* 1991). The region in which LC1 and LC3 of higher vertebrates share an identical amino acid sequence is boxed in Fig. 4. There were many substitutions between carp LC1 and LC3 in this region, as had already been suggested by their nucleotide sequences (see Fig. 3).

### *N-terminal amino acid sequences of carp myosin alkali light chains*

Tryptic digests of carp myosin LC1 and LC3 contained at least seven and five components, respectively, as shown in SDS–PAGE (Fig. 5A). Undigested bands of LC1 and LC3 did not give any information on their N-terminal amino acids, probably as a result of trimethylation (Henry *et al.* 1982) and acetylation (Frank and Weeds, 1974), respectively. All tryptic digests had identical amino acid sequences with those deduced from their respective nucleotide sequences (Fig. 5B, underlined sequences in Fig. 4). In addition, the second band produced by tryptic digestion of LC1 showed two amino acids, Ala and Pro, in the fifth position from the N terminus (Figs 5, 6). The deduced amino acids in this position of LC1a and LC1b were also Ala and Pro, respectively (see Fig. 4). These data indicate that both LC1a and LC1b are expressed *in vivo*.

### *Southern blot analysis of carp myosin alkali light chain genes*

Comparison of cDNA nucleotide and amino acid sequences between carp myosin LC1 and LC3 suggests that they are produced from different genes. In order to investigate the genomic organization of carp myosin alkali light chains, Southern blot analysis was performed using carp hepatopancreas genomic DNA. DNA fragments specific to carp LC1a and LC3 were used as probes. These were derived from the 3′ non-coding region, extending from the nucleotide 3′ to the stop codon to that immediately before a poly A tail and share 55 % sequence homology. The specificity of these probes was confirmed by dot-blot analysis; no apparent discrimination was observed between LC1a and LC1b cDNA clones (data not shown). Fig. 7 shows hybridization patterns in Southern blot analysis with several restriction enzymes. The patterns obtained with LC1- and LC3-specific probes were clearly different from each other, confirming that carp myosin LC1 and LC3 are encoded by different genes.

### *Northern blot analysis of carp myosin alkali light chains*

Crockford and Johnston (1990) claimed that the molar ratio of LC3 to LC1 in carp changed during thermal acclimation. They found that the ratios in 8 °C- and 20 °C-acclimated carp were 2.29 and 2.87, respectively, which were significantly different ($P$<0.002). In the present study, we determined the mRNA levels of LC1 and LC3 in 10 °C- and 30 °C-acclimated carp and compared them with those of α-actin mRNA (Fig. 8). No apparent changes in the level of α-actin mRNA were observed following temperature acclimation. In contrast, the LC3:LC1 mRNA ratio of the 10 °C-acclimated carp was 3.10, whereas that of the 30 °C-acclimated carp was 3.93, the difference being significant at $P$<0.05.

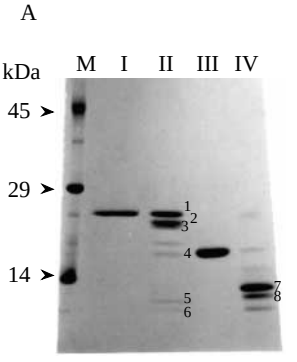


Fig. 5. SDS–PAGE patterns (A) and N-terminal amino acid sequences (B) of carp myosin alkali light chains and their tryptic digests. Lanes I and III are purified LC1 and LC3, respectively, and lanes II and IV are tryptic digests of LC1 and LC3, respectively. The protein bands of the tryptic digests are numbered to correlate their N-terminal amino acid sequences in B with those deduced from cDNA nucleotide sequences indicated by LC1a and LC3 (see Fig. 4). Lane M contained marker proteins.

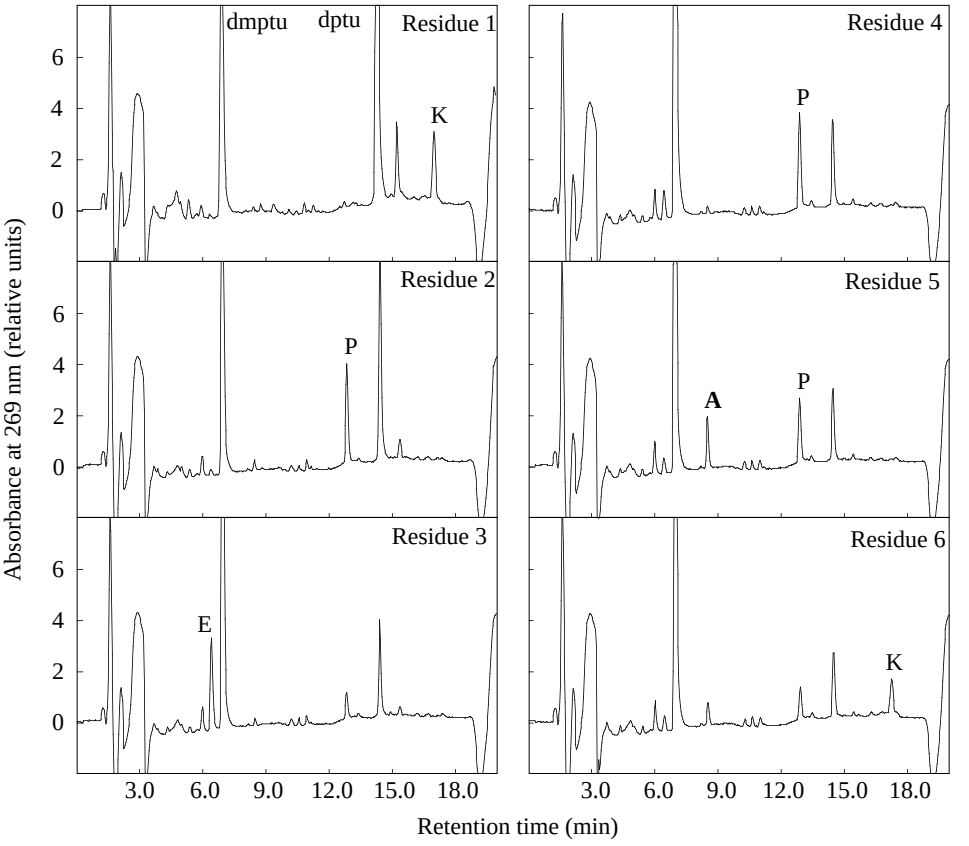


Fig. 6. N-terminal amino acid sequencing for the second high-molecular-mass tryptic digest from LC1. LC1 was digested with trypsin, and the product with the second-highest molecular mass (band 2 in Fig. 5) was subjected to N-terminal amino acid sequence analysis as described in Materials and methods. Note that both alanine (A) and proline (P) are found in the fifth position from the N terminus. Byproducts from Edman degradation are *N*′,*N*-dimethyl-*N*′-phenylthiourea (dmptu) and *N*′,*N*-diphenylthiourea (dptu). K, lysine; E, glutamic acid.

## Discussion

It is known that the fast skeletal myosin alkali light chains, LC1 and LC3, of mammalian and avian species are produced from a single gene by alternative transcription and two modes of splicing (Nabeshima *et al.* 1984; Robert *et al.* 1984; Periasamy *et al.* 1984). The myosin LC3 sequence is identical with that of the C-terminal two-thirds of LC1. However, in mullet, differences in the sequences of LC1 and LC3 have been reported to exist in the C-terminal region, in addition to the so-called difference peptide in the N-terminal region of LC1 (Dalla Libera *et al.* 1991).

In this study, cDNA clones encoding carp fast skeletal myosin alkali light chains were isolated and their nucleotide and deduced amino acid sequences determined. Two types of cDNA clones encoding carp LC1 were found and termed LC1a and LC1b. Their nucleotide sequences resembled each other, showing 92 % homology, but differences were found in both coding and non-coding regions. N-terminal amino acid sequences of tryptic digests from purified carp LC1 also revealed the presence of LC1 isoforms at the protein level. Crockford *et al.* (1995) reported that fast muscle from the tropical fish *Oreochromis andersonii* contains two myosin LC1 isoforms. Breeding experiments confirmed that the different light chains in *O. andersonii* were encoded by alleles of a single gene. Evidence was obtained that allelic variations in LC1 were associated with differences in the maximum contraction velocity of fast muscle fibres, suggesting that the variation has some functional significance *in vivo*. Multiple LC1s have also been described in chicken fast muscle (Rushbrook and Somes, 1985) and in the white muscle of Arctic charr (*Salvelinus alpinus*)

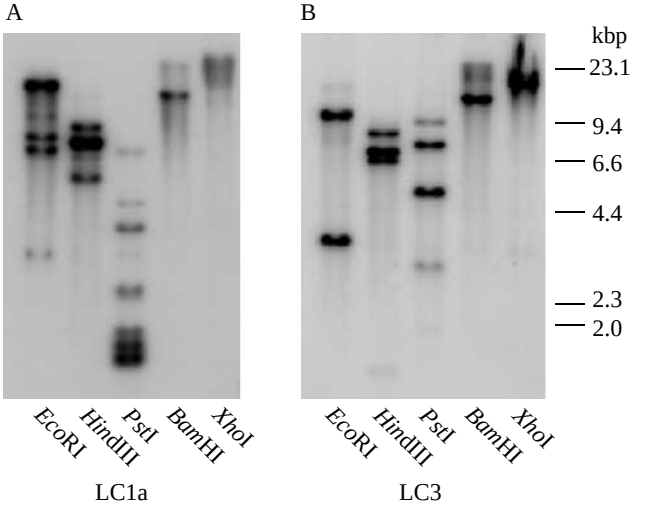


Fig. 7. Southern blot patterns of carp myosin alkali light chain genes. Carp hepatopancreas genomic DNA (10 µg) was digested with restriction endonucleases, including *Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI and *Xho*I. Digests were then blotted onto nylon membranes and hybridized with cDNA fragments specific to LC1a (A) and LC3 (B). No specific cDNA fragments which distinguish LC1a and LC1b were established because of the marked similarity in their nucleotide sequences. kbp, kilobase pairs.

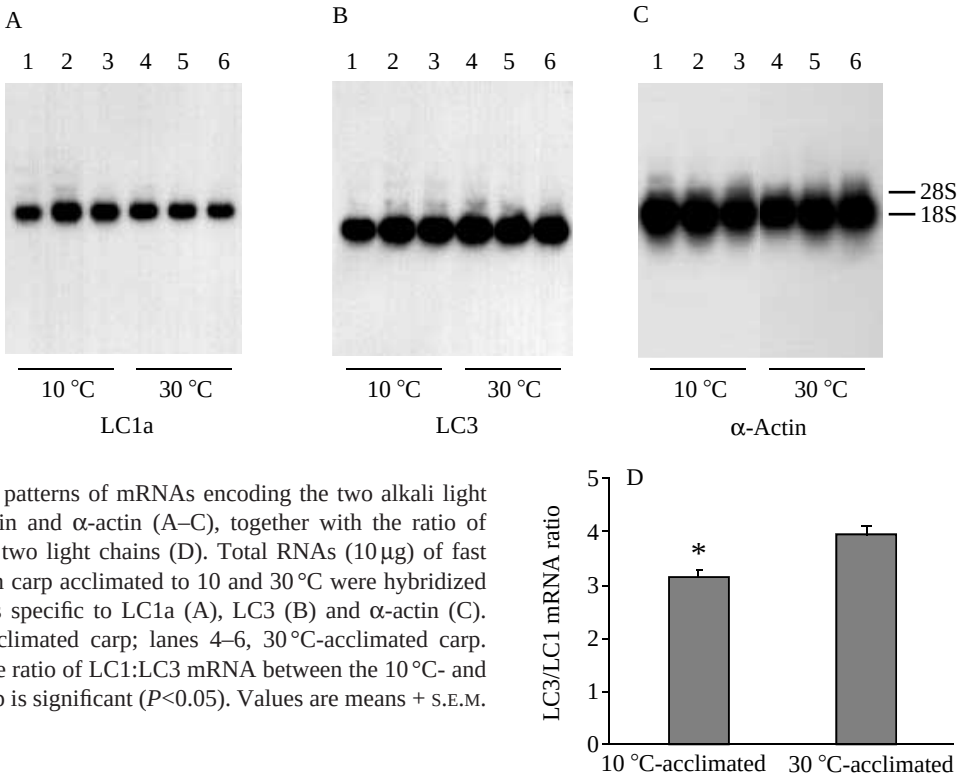


Fig. 8. Northern blot patterns of mRNAs encoding the two alkali light chains of carp myosin and α-actin (A–C), together with the ratio of mRNA levels of the two light chains (D). Total RNAs (10 μg) of fast skeletal muscles from carp acclimated to 10 and 30 °C were hybridized with DNA fragments specific to LC1a (A), LC3 (B) and α-actin (C). Lanes 1–3, 10 °C-acclimated carp; lanes 4–6, 30 °C-acclimated carp. *The difference in the ratio of LC1:LC3 mRNA between the 10 °C- and 30 °C-acclimated carp is significant ($P$<0.05). Values are means + S.E.M. ($N$=3).

(Martinez and Christiansen, 1994). In the latter case, variations of LC1 expression were found between isolated Arctic charr populations. No isoform clones encoding LC3 were isolated from our 10 °C- and 30 °C-acclimated carp cDNA libraries.

Comparison of the nucleotide sequence between carp LC1 and LC3 cDNA clones revealed that differences occurred in all the overlapping areas, including both coding and non-coding regions. Comparisons of the amino acid sequences of LC1 and LC3 also indicate that differences are found over the C-terminal region, where LC1 and LC3 of higher vertebrates share an identical sequence. These results with carp are consistent with those reported for mullet (Dalla Libera *et al.* 1991). Southern hybridization patterns using probes specific to LC1 and LC3 were clearly distinct, confirming that fish myosin alkali light chains are produced from different genes.

It is known that cyprinid fish change the myofibrillar ATPase activity in fast skeletal muscle during temperature acclimation (Johnston *et al.* 1975; Heap *et al.* 1985). Acclimation to low temperature results in an increase in myofibrillar ATPase activity. The expression of different myosin heavy chain isoforms is responsible for these changes in carp (Hwang *et al.* 1991; Watabe *et al.* 1992; Guo *et al.* 1994). Acclimation-temperature-dependent types of cDNAs encoding myosin heavy chain isoforms have recently been cloned from 10 °C- and 30 °C-acclimated carp (Watabe *et al.* 1995*b*). In addition, Crockford and Johnston (1990) found that the molar ratio of LC3 to LC1 in carp also changed during thermal acclimation. The ratio for 8 °C-acclimated carp was 2.29, whereas that for 20 °C-acclimated fish was 2.87. Northern blot analysis in our study showed the same tendency and revealed that the molar ratio of LC3 to LC1 increased at the level of mRNA accumulation following warm temperature acclimation of carp. Weeds and Taylor (1975) found that the Vmax of actin-activated $Mg^{2+}$-ATPase activity of rabbit fast skeletal S1 containing LC1 was approximately half that for S1 containing LC3, although the ionic strength in the assay medium was much lower than the physiological level. In addition, Lowey *et al.* (1993) have recently shown that rabbit myosin containing LC3 moves on actin filaments approximately 1.5 times as fast as the LC1-containing counterpart. The same relationship in actin-activated S1 ATPase activity has been observed for fish myosin isoforms containing LC1 and LC3 (Ochiai *et al.* 1989*a*). However, muscle fibres in cold-acclimated carp have a lower LC3 content and yet have a significantly higher maximum contraction velocity at low temperature than fibres from warm-acclimated fish (Johnston *et al.* 1985, 1990), suggesting that the effects of the myosin heavy chain isoform present predominate (Hwang *et al.* 1990; Guo *et al.* 1994).

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