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Molecular Cloning of the Myosin Light Chain-2 cDNA of *Gryllotalpa orientalis*

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We describe here the cloning and characterization of a cDNA encoding a putative myosin light chain-2 (MLC-2) from the mole cricket, *Gryllotalpa orientalis*. The *G. orientalis* MLC-2 cDNA sequences comprised of 615 bp with 205 amino acid residues with a calculated molecular weight of approximately 23 kDa. The deduced protein sequence of *G. orientalis* MLC-2 cDNA showed 64% and 54% identity to *Drosophila melanogaster* MLC-2 and *D. yakuba* MLC-2, respectively. Northern blot analysis confirmed the muscle-specific expression of *G. orientalis* MLC-2.

Key words: Insect, Oriental mole cricket, *Gryllotalpa orientalis*, cDNA sequences, Muscle, Myosin light chain-2

Introduction

Myosin is a hexameric protein which forms the core of the thick filaments of muscle. All myosin filaments are composed of two heavy chains and two pairs of light chains. Force production in muscle requires the formation of mechanically strained, elastic cross-bridges between myosin- and actin-containing filaments. These cross-bridges are composed of the globular heads of the myosin heavy chain subunits and their associated light chains (Adelstein and Eisenberg, 1980).

The myosin light chain-2 (MLC-2) is one of the pairs of myosin light chains. MLC-2 proteins are members of the troponin C superfamily (Barker *et al.*, 1978). MLC-2 is associated with the globular head of the myosin heavy chain and is believed to modulate the actin-activated myosin-linked Mg^{2+}-ATPase (Sweeney and Stull, 1990).

In insects, MLC-2 was previously characterized in *Drosophila* (Parker et al., 1985; Toffenetti *et al.*, 1987; Warmke *et al.*, 1992). A null mutation of the MLC-2 gene in *Drosophila* resulted in reduced accumulation of MLC-2 protein in indirect flight muscle (IFM), and the wild-type MLC-2 stoichometry is required for normal myofilament lattice alignment during IFM myogenesis and for normal IFM function as assayed by flight behavior, wing beat frequency and single fiber mechanics (Warmke *et al.*, 1992).

We have previously constructed the cDNA library using *Gryllotalpa orientalis* whole bodies for the genetic information of the mole cricket (Kim *et al.*, 2002). In this study, we report the cDNA sequence for putative MLC-2 protein from *G. orientalis*. The protein sequence is compared with other MLC-2 protein sequences. We describe the muscle-specific expression of the *G. orientalis* MLC-2 gene.

Materials and Methods

Animals

The mole cricket, *Gryllotalpa orientalis* (Burmeister), was collected in Kimhe city, Korea (Kim *et al.*, 2002).

cDNA library screening , nucleotide sequencing and data analysis

A cDNA library (Kim *et al.*, 2002) was constructed using whole bodies of *G. orientalis*. Sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega, Madison, WI). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences

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were compared using the DNASIS and BLAST programs provided by the NCBI (www.ncbi.nlm.nih.gov). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program (www.ncbi.nlm.nih.gov/BLAST).

MacVector (ver. 6.5, Oxford Molecular Ltd.) was used to align the amino acid sequences of MLC-2. Including the two GenBank-registered MLC-2 amino acid sequences, phylogenetic analysis was performed using PAUP* (Phylogenetic Analysis using Parsimony) version 4.0 (Swofford, 2000). The accession numbers of the sequences in the GenBank are: *G. orientalis* (AY700062; this study), *Drosophila melanogaster* (P18432; Toffenetti *et al.*, 1987), and *Drosophila yakuba* (AY231768; Domazet-Loso and Tautz, 2003).

RNA isolation and Northern blot analysis

The *G. orientalis* was dissected under the Stereo-microscope (Zeiss, Jena, Germany), individual samples such as fat body, midgut, muscle, and epidermis were harvested, and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNA was isolated from fat body, midgut, muscle, and epidermis of the *G. orientalis* using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from *G. orientalis* was denatured by glyoxalation (McMaster and Carmichael 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell) and hybridized at 42°C with a probe in a hybridization buffer containing 5 × SSC, 5 × Denhardts solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. The 915 bp MLC-2 cDNA clone was labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) for use as a probe for hybridization. After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C and exposed to autoradiography film.

Results and Discussion

cDNA library was constructed using whole bodies of *G. orientalis*. Sequencing of randomly selected clones harboring cDNA inserts was performed to generate *G. orientalis* ESTs. Of these ESTs, one clone, which is 915 bp long had a full-length coding sequence similar to that of previously reported myosin light chain-2 (MLC-2) proteins. The nucleotide and deduced amino acid sequences of *G. orientalis* MLC-2 protein are presented in Fig. 1. The nucleotide and deduced amino acid sequences of cDNA encoding a putative member of the insect MLC-2

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                                GGACAGACAACACCAGTAGGAGGTCCTTTCTTCTTCGACACACACA
1   ATGGCGGACAAGGAAAAGAAGAAGAAGAAGAAGTCCACCAAGGAGGAGGCACCAGCAGAA
1   M  A  D  K  E  K  K  K  K  K  K  S  T  K  E  E  A  P  A  E
61  GCGGCGCCCGCCGCCGAAGCACCCGCCCCGGCCGCTGACTCCAACCGCCAGAGCTCCCGT
21  A  A  P  A  A  E  A  P  A  P  A  A  D  S  N  R  Q  S  S  R
121 GGCTCCAGGAAGGCCAAGCGCACCGGTAGCAATGTCTTCTCCATGTTCTCCCAGAAGCAG
41  G  S  R  K  A  K  R  T  G  S  N  V  F  S  M  F  S  Q  K  Q
181 GTCGCCGAGTTCAAGGAGGCCTTCCAGCTGATGGACCATGACAAAGATGGTATCATCAAC
61  V  A  E  F  K  E  A  F  Q  L  M  D  H  D  K  D  G  I  I  N
241 AAGAACGACCTTCGTGCCACCTTTGACAGCTTGGGCCGTCTCGCCAGCGACAAGGAGTTG
81  K  N  D  L  R  A  T  F  D  S  L  G  R  L  A  S  D  K  E  L
301 GACGAGATGGTCAGCGAGGCCCCTGGACCCATCAACTTCACCCAGCTCCTCACTCTCTTC
101 D  E  M  V  S  E  A  P  G  P  I  N  F  T  Q  L  L  T  L  F
361 GCTGCCAGAATGTCTGGAGGTTCTGATGATGATGATGTCGTCATCAACGCTTTCAAAACA
121 A  A  R  M  S  G  G  S  D  D  D  D  V  V  I  N  A  F  K  T
421 TTCGATGACAACGGCAGAATCGATAGCGAGAGGCTGCGACACGCTCTCATGACCTGGGGC
141 F  D  D  N  G  R  I  D  S  E  R  L  R  H  A  L  M  T  W  G
481 GACAAGTTCTCCGCCGATGAGGTGGACGACGCCTACGAGCAGATGGTCATCGACGACAAA
161 D  K  F  S  A  D  E  V  D  D  A  Y  E  Q  M  V  I  D  D  K
541 GGCTTCATCGACACCCAGAAGCTCATCACCATGCTGACGGCCAGCGCCGAGGAGGAAGAA
181 G  F  I  D  T  Q  K  L  I  T  M  L  T  A  S  A  E  E  E  E
601 GAAGGCGAGGCTGCCTAAACCTTCCACTAGCCTCATTAGCCACCCACCACCAGCAGGCCG
201 E  G  E  A  A  *
661 TCGCCTCCCCAGCCGAGACCGGAGTCCGCGCCTTCCTTCAGACTCCTATCTTCCGCGTCA
721 ACTCTCGGTGGAGGCCATTCTGCGGAGCTTGACTCATTAAAGCGCTCCACTATCACTTAA
781 GTACATAAACTGATTCACATCTTGTCCATAGCACTATCTGTATCGAGTGAATCTAAGATT
841 ATAAATATCCGTTCATGAAAAAAAAAAAA
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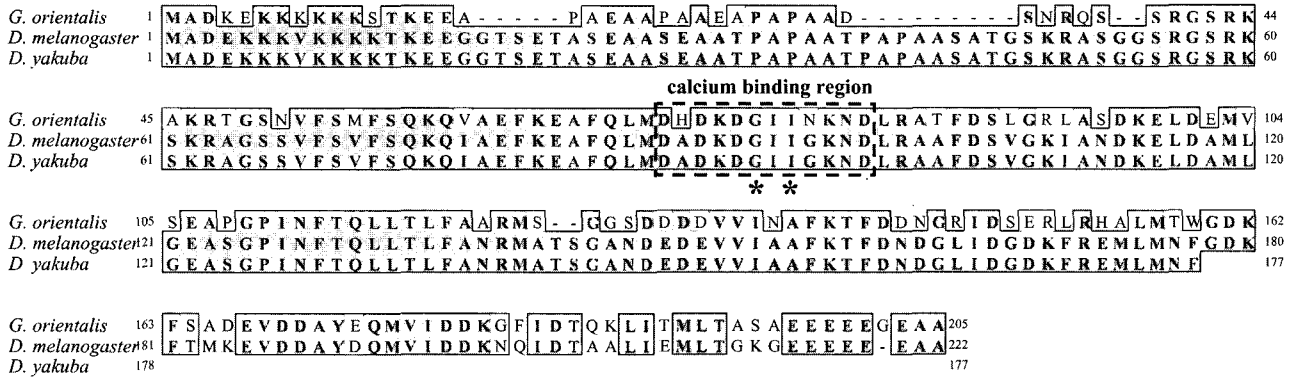
Fig. 1. The nucleotide and deduced amino acid sequences of *G. orientalis* MLC-2 cDNA. The start codon ofATG is boxed and the termination codon is shown by asterisk. The polyadenylation signal AATAAA is underlined. This cDNA sequence has been deposited in GenBank under accession number AY700062.

protein revealed that the cDNA sequences comprised of 615 bp with 205 amino acid residues with a calculated molecular weight of approximately 23 kDa.

A multiple sequence alignment of the deduced protein sequence of *G. orientalis* MLC-2 cDNA with other insect MLC-2 protein sequences is shown in Fig. 2. As shown in Fig. 2A, *D. melanogaster* MLC-2 comprised of 222 amino acid residues while D. yakuba MLC-2 with 177 amino acid residues. The three of the insect MLC-2 proteins possess conserved calcium binding region (Parker *et al.*, 1985). Furthermore, glycine (G) and isoleucine (I) residues, which are often found in Ca^{2+}-binding domains (Kretsinger, 1980), are well conserved in all insect MLC-2 proteins. The *G. orientalis* MLC-2 protein showed 64% and 54% protein sequence identity to the *D. melanogaster* MLC-2 and *D. yakuba* MLC-2, respectively (Fig. 2B).

MLC-2 proteins are members of the troponin C superfamily. This group of evolutionally related proteins all contains one or several domains designed as EF hands (Barker *et al.*, 1978). This domain provides a structure capable of binding a calcium ion by chelation. Members of the troponin C superfamily are believed to have evolved from an ancestral Ca^{2+}-binding protein with a single EF. The tissue specific nature of *G. orientalis* MLC-2 expression was determined from fat body, midgut, muscle, and epidermis by Northern blot analysis (Fig. 3). *G. orientalis*

(A)



(B)

Species	GenBank No.	Percent similarity 1	2	3
G. orientalis	AY70062		73	62
D. melanogaster	P18432	64		79
D. yakuba	AY231768	54	79	

Percent identity

Fig. 2. Comparison of the deduced amino acid sequence of *G. orientalis* MLC-2 with the *D. melanogaster* MLC-2 and *D. yakuba* MLC-2. (A) Alignment of deduced amino acid sequences of *G. orientalis* MLC-2 with two insect MLC-2 protein sequences. Residues are numbered according to the aligned MLC-2 sequences, and invariant residues are shaded black. The calcium binding domain is shown in dotted box. (B) Pairwise identities and similarities of the deduced amino acid sequence of *G. orientalis* MLC-2 cDNA with the *D. melanogaster* MLC-2 and *D. yakuba* MLC-2.

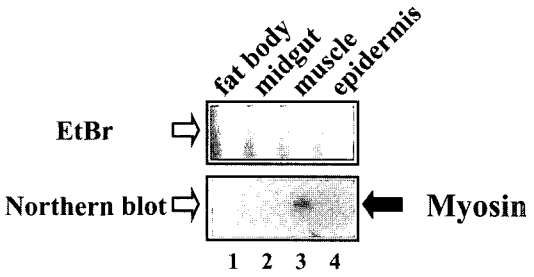


Fig. 3. Northern blot analysis of the *G. orientalis* MLC-2 messages. Total RNA was isolated from fat body (lane 1), midgut (lane 2), muscle (lane 3), and epidermis (lane 4). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred onto a nylon membrane, and hybridized with the appropriate radiolabelled probe (lower panel). *G. orientalis* MLC-2 transcripts are indicated by arrow on the right side of the panel.

MLC-2 was found to be expressed only in muscle of *G. orientalis*, evidencing the muscle as a specific site for MLC-2 synthesis. Our present result is in good agreement with the previous finding in that the MLC-2 protein mRNAs have been detected in the muscle of *Drosophila* (Parker *et al.*, 1985).

In conclusion, we have cloned a novel MLC-2 cDNA from the mole cricket, *G. orientalis*. We are now interested in the molecular information of MLC-2 cDNA of insect origin, which may help to further our understanding of insect myogenesis.

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