Thesis for the degree of Master of Science

Myosin heavy chains and twelve actins differentially expressed in muscle fiber types from American Lobster (*Homarus americanus*)

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바닷가재 (*Homarus americanus*) 의 근 섬유의 종류에 따른 Myosin heavy chains 과 12 개의 actin 의 발현에 관한연구

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TABLE OF CONTENTS

LIST OF FIGURES.....iii

LIST OF TABLES.....v

ABSTRACT.....vi

INTRODUCTION.....1

MATERIALS AND METHODS.....5

1. Experimental animal.....5

2. Differential display reverse transcription polymerase chain reaction.....5

3-1. Cloning of HA-Actins.....7

3-2. Cloning of myosin heavy chain (MHC).....8

4-1. Expression of HA-Actins.....12

4-2. Expression of MHC.....13

5. Data analysis and statistics.....14

RESULTS AND DISCUSSION

1. Isolation of twelve actin cDNAs and MHC from lobster.....15

Actin.....15

MHC.....18

2. Structural analysis.....20

Actin.....20

MHC.....25

3. Expression study.....33

Actin.....33

MHC.....36

REFERENCES.....46

ACKNOWLEDGEMENTS.....56

LIST OF FIGURES

Fig. 1. Result of DDRT-PCR. PCR products from crusher (cr) and cutter (cu) were separated on the 1.5 %agarose gel. The products from 17 and 111 were identified as actin and MHC.....9

Fig. 2. The nucleotide and deduced amino acid sequences of HA-F2MHC. Box shows a repeating 28-amino acid sequence pattern. An asterisk (*) indicates the stop codon…..19

Fig. 3. Comparison of the amino acid sequences of actins from American lobster (*Homarus americanus*).....28

Fig. 4. . Phylogenetic diagram of the different actins based on the amino acid sequence similarity.....31

Fig. 5. Expression of HA-ACTIN (A) and HA-MHC (B) genes in various tissues.....38

Fig. 6. Relative copy numbers of Ha-actins (A) and HA-MHCs (B) in each tissue of *H. americanus*.....40

Fig. 7. Component ratio of HA-Actins within the same tissue throughout relative copy

numbers of HA-Actins in *H. americanus*.....42

LIST OF TABLES

Table 1. Primer list of actins (A) and MHCs (B).....10

Table 2. Composition of actin cDNAs.....17

Table 3. Comparison of amino acid sequence of twelve actins from American lobster...27

Table 4. Relative copy number of twelve actin (A) and (B) mRNAs.....43

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Abstract

Unlike mammals, crustacean muscle fibers dynamically change according to the developmental and molting stage. It is important to understand molecular mechanism of its plasticity and there are many possible applications for medical and aquaculture industry. In present study, twelve actins and one myosin heavy chain (MHC) cDNAs were isolated from the different tissues of American lobster, *Homarus americanus*; eight skeletal muscle type actins (HA-ActinSK), one cardiac muscle type actin (HA-ActinHT), three cytoplasmic type actins (HA-ActinCT) and fast type MHC. End-point RT-PCR and quantitative PCR results showed that HA-ActinSK1 and SK2 are major actins of the crusher claw muscle and HA-ActinSK3, SK4 and SK5 are main actins of cutter claw muscle. Deep abdominal (DA) muscles were mainly composed of HA-ActinSK5 and SK8

and DA flexor contains additional HA-ActinSK7. HA-ActinSK7 may be important component to distinguish between DA flexor and extensor muscle. HA-ActinHT1 is exclusively expressed in the heart muscle. Three cytoplasmic actins, HA-ActinCT1, CT2 and CT3 were predominantly expressed in the hepatopancreas. Isolated MHC was strongly expressed in the fast type muscles (cutter, DAF, DAE). Amino acid sequence alignment and phylogenetic analysis results suggest that actins and MHCs appear to be functionally active and exhibit each muscle fiber type charaters. In addition, each gene was highly related to the tissue where they expressed, suggesting that amino acid sequence of each is strongly related with functional or structural character of each tissue type.

Introduction

American lobsters are good model system to understanding muscle dynamics because their muscle fibers dramatically change according to the developmental and molting stages. Specially, claw muscle of lobster are dimorphic, with a large crusher claw and a slender cutter claw (Govind, 1984, 1992; Govind et al., 1987). Claw muscles of crusher are composed of slow fibers, whereas cutter muscle are almost composed of fast fiber. This dichotomy appeared during the juvenile stages of development, over a series of generally 13 molt cycles (Govind, 1984, 1992; Govind etal., 1987). After the fifth molt stage, the muscle fibers differ a transformation into almost fast fibers of the cutter claw, and slow fibers of the crusher. Crustacean muscles are classified as fast, slow twitch (S1), and slow tonic (S2). The previously studies of myofibrillar protein found that different muscle tissue is composed of different myofibrillar proteins (Mykles 1985a; Mykles 1985b). Fast fibers are detected mostly from the cutter claw and deep abdominal flexor and extensor muscles. S1 fibers are mainly found in crusher claw closer and present in ventral musculature of the cutter claw closer. S2 fibers are present in the distal fibers of the cutter claw closer, claw and leg opener, and as well as superficial abdominal flexors and extensors. Medler et al. measured

expression patterns and levels of different subtypes of MHCs, tropomyosin, and actin according to fiber types using both real-time PCR and PAGE technique (Medler et al. 2003; Medler et al. 2007). He found correlation in expression of each fiber protein among those types of muscle fiber. He also found that single muscle fibers often express multiple isoforms of myofibrillar proteins (Medler et al. 2004). However, results of his study had only a limited understanding because of lack of nucleotide information of different subtypes of muscle fiber genes. In addition, although he found positive correlation between actin and sMHC expression and predominant expression in crusher muscle compared with that in cutter muscle. These results suggested possible more actin genes and each has its own partner fiber proteins including myosin heavy chain and tropomyosines.

Myosin heavy chains (MHCs) are actin-based molecular motors that convert chemical energy released from the hydrolysis of ATP into mechanical force in eukaryotic cells. In mammals, eight sarcomeric MHC genes, two cardiac and six skeletal are found in tightly linked clusters on human chromosomes 14 and 17 (Saez et al., 1987; Qin et al., 1990; Leinwand et al., 1983; Edwards et al., 1985; Yoon et al., 1992), mouse 14 and 11 (Gulick et al., 1991; Leinwand et al., 1983; Weydert et al., 1985), and rat 14 and 10 (Mahdavi et al., 1984; Remmers et al., 1992), respectively.

Actins are one of the most conserved proteins found in all eukaryotic cells. In spite of a high degree of homology, individual actins exhibit various genomic structure and patterns of expression according to species, tissue, and developmental stages. These complex actins participate in many important cellular functions, including muscle contraction, cell motility, cell division, vesicle and organelle movement, and the establishment and maintenance of cell junction and cell shape (Rubenstein 1990). Mammals have six different isoforms actins including two striated muscle (α-skeletal and α-cardiac), two smooth muscle (α-aortic and γ-enetric), and two cytosolic (β- and γ-) (Vandekerckhove et al. 1978). Teleost fish have nine actin genes; six muscle-type and three cytosolic acitins (Venkatesh et al., 1996). Invertebrate actins are more complicated than vertebrate. Cnidaria has two or three actins, most of which shows high homology to vertebrae cytoplasmic actins (Fisher et al. 1989; Fukuda et al. 2002). 6-10 cytoplasmic actins and only one muscle actin gene were isolated in echinoids (Cooper et al. 1982; Fang et al. 1994). Southern blot analysis suggests that 12-15 actin genes in the bivalve genome(Parwary et al. 1996). *D. melanogaster* has six actin genes and their expression and functions change according to specific tissue types (Fyrberg et al. 1980; Fyrberg et al. 1998). Among crustacean Artemia has 8-10 genes were identified and land crab, *Gecarcinus lateralis*, appear seven or eight action genes (Macias et al. 1990; Ortega et al. 1992;

Varadaraj et al. 1996). Although there have been numerous number of actin genes reported, it is still not easy to clone gene for each fiber protein because the size of those proteins is too similar to distinguish just by PAGE analysis and nucleotide sequences are too similar to isolate each transcript. In present study, we isolated twelve actins and one myosin heavy chain in various tissues from lobster by combination of differential display RT-PCR (DDRT-PCR) and using modified conventional cloning PCR technique. Their structure and expression study suggests that actin and MHC is strongly related to expression pattern.

Materials and Methods

1. Experimental animal

live adult lobsters were purchased from seafood market and cultured in a tankwith circulation seawater (6 ˚C). They were provided small shrimps for a week 12 h day and night period. Various tissues were isolated from each lobster and were stored in -80 ˚C deep freezer until they were used for total RNAs purification.

2. Differential display reverse transcription polymerase chain reaction

Total RNAs were isolated from crusher and cutter using the Trizol reagent according to the manufacturer's instructions (Invitogen, USA) and quantified with a ND-1000 NanoDrop UV spectrophotometeter (Nanodrop Technologies, Inc.). In order to identify differentially expressed genes, we used the differentially expressed gene system (GeneFishing^{TM} DEG Premix kit; Seegene Inc., korea). First-strandcDNA was synthesized in a reaction containing reverse transcriptase

and dT-ACP1 (Annealing Control Primer). Total RNA samples were treated with DNase Ⅰ (TaKaRa, Japan) for 20 min at 37 ˚C to remove any genomic DNA contaminating the RNA samples. A 12-μl mixture containing 3 μg total RNA, 1 μl 20 μM dT-ACP1 and 4 μl dNTPs (2.5mM) was heated to 70 ˚C for 5 min and chilled on ice for 2 min. First-strand buffer (5x, 4 μl), 2 μl 0.1 M DTT and 1 μl RNase out were added to the mixture, which was incubated at 42 ˚C for 2 min. Superscript Ⅱ reverse transcriptase (1 μl) was added and then the mixture was incubated at 42 ˚C for 50 min. Differential display reverse transcription polymerase chain reaction (DDRT-PCR) was carried out with annealing control primer (ACP) and dT-ACP2 primer pairs. A final reaction volume of 20 μl contain 3 μl cDNA (300ng), 2 μl 5 μM arbitrary ACP (ACP1-ACP120), 1 μl dT-ACP2 (10 μM), 10 μl 2X Master Mix and 4 μl distilled water. The PCR was carried out the following conditions: 1 cycle at 94 ˚C for 5 min, 50 ˚C for 3 min, 72 ˚C for 1min, after, 40 cycle of 94 ˚C for 40 s, 65 ˚C for 40 s, 72 ˚C for 40 s, and 5 min final extension at 72 ˚C. PCR products were identified with 1.5% agarose gel electrophoresis stained with ethidium bromide. The PCR products expressed differently between crusher and cutter were isolated from gel slices using Gel Extraction Kit (Bioneer, Korea), ligated into TA plasmid vector with the pGEM-T Easy Cloning Kit (Promega, USA), and transformed into XL1-blue competent cell. cDNAs were sequenced with an automated DNA sequencer (ABI Biosystem,

USA). The nucleotide sequence similarities were analyzed by BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

3-1 Cloning of HA-Actins

From the result of DD RT-PCR, we identified new actin in cutter (Fig.1). To obtain each tissue specific actins, total RNAs from the crusher, cutter, deep abdominal flexor (DAF), deep abdominal extensor (DAE), heart and hepatopacreas were purified using the Trizol reagent according to the manufacturer's instructions (Invitogen. USA). First, we modified 3' RACE to obtain cDNA sequences contain variable 3'UTR region for each actin gene. First-strand cDNA was synthesized using DEG-3RACE primer (Table 1). PCR was carried out with degenerated forward primers and DEG linker primers. PCR reaction was carried out in a final volume of 30 μl containing cDNA, degeneracy primer, specific primer and Takara Ex Taq polymerase, dNTP(2.5mM) and 10X buffer (Takara, Japan). PCR conditions were 1 min at 94 ˚C, followed by 40 cycles at 94 ˚C for 1 min, 50 ˚C for 30 sec, and 72 ˚C for 30 sec. PCR products were identified with 1.5% agarose gel electrophoresis stained with ethidium bromide. The PCR products the crusher, cutter, deep abdominal flexor (DAF), deep abdominal extensor (DAE), heart and hepatopacreas were isolated from

gel slices using Gel Extraction Kit (Bioneer. Korea), ligated into TA plasmid vector with the pGEM-T Easy Cloning Kit (Promega, USA), and transformed into XL1-blue competent cell. cDNAs were sequenced with an automated DNA sequencer (ABI Biosystem, USA). For obtained full ORF sequence, RT-PCR was carried out with forward degeneracy primer including start codon (ATG) and 3'UTR specific reverse primer for each actin gene (Table 1). PCR conditions were 1 min 94 ˚C, next to 40 cycles at 94 ˚C for 1min, 50 ˚C for 30 sec, 72 ˚C for 1min 30 sec and post-extention at 72 ˚C for 5 min. Full ORF sequences were obtained by combining two sequence using the BLAST 2 SEQUENCES program (http://blast.ncbi.nlm. nih.gov/bl2seq/wblast2.cgi) and reconfirmed the single transcript by PCR using specific forward and reverse primers.

3-2. Cloning of myosin heavy chain (MHC)

Partial sequence of new MHC was identified from DD RT-PCR result in cutter (Fig.1). To obtain 5' region, we carried out RT-PCR with degeneracy forward and reverse specific primers (Table 1). Primary PCR reaction was carried out in a final volume of 30ul containing cDNA, linked primer, degeneracy primer and Takara Ex Taq polymerase, dNTP(2.5mM) and 10X buffer (Takara. Japan). The PCR condition was 1 cycle of 94 ˚C 1 min followed by 40 cycles of 94 ˚C for 30 s,

55 ˚C for 30, 72 ˚C for 1 min 30 s, and final extention time 72 ˚C 5 min. Nested PCR condition and mixtures were carried out same to the primary PCR. PCR product was then isolated from gel slices using Gel Extraction Kit (Bioneer. Korea), ligated into TA plasmid vector with the pGEM-T Easy Cloning Kit (Promega, USA), and transformed into XL1-blue competent cell. cDNAs were sequenced with an automated DNA sequencer (ABI Biosystem, USA).

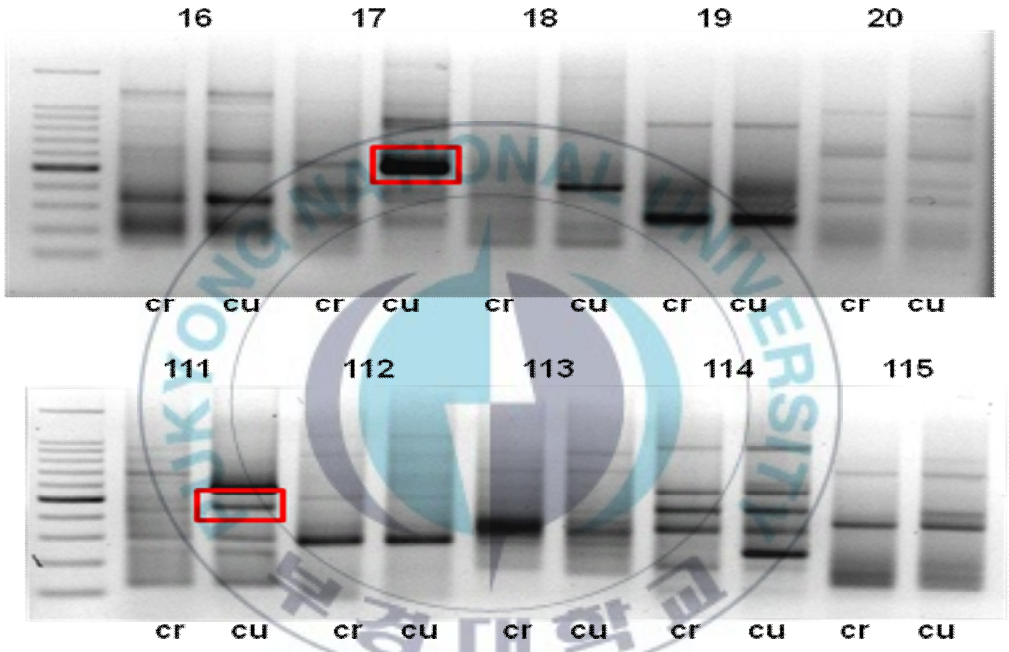


Fig. 1. Result of DDRT-PCR. PCR products from crusher (cr) and cutter (cu) were separated on the 1.5 % agarose gel. The products from 17 and 111 were identified as actin and MHC.

Table 1. Primer list of actins (A) and MHCs (B)

A

Name	Sequence	Description
ACTIN F20	5'-ATGTGYGAYGANGANG-3'	Degeneracy FWD primer for RT-PCR
ACTIN F41	5'-TCYGGYGGYACCACCATGAC-3'	First degeneracy FWD primer for 3'RACE
ACTIN F42	5'-GGYGGYACCACCATGACCC-3'	Second degeneracy FWD primer for 3'RACE
DEG-3RACE	5'-CTGTGAATGCTGCGACTACGAdT(16)-3'	cDNA synthesis primer for 3'RACE
DEG-3RACE-1	5'-TGTGAATGCTGCGACTAC-3'	First RVS primer for 3'RACE
DEG-ACP3	5'-TGAATGCTGCGACTACGA-3'	Second RVS primer for 3'RACE
HA-SK1 F1	5'-CATTGCTGCTGCCTCTTCT-3'	Specific FWD primer for RT-PCR
HA-SK1 R1	5'-TGCTGTTGTGACTCATTATCT-3'	Specific RVS primer for RT-PCR
HA-SK2 F1	5'-GATGAACATTGCTGCTGCA-3'	Specific FWD primer for RT-PCR
HA-SK2 R1	5'-ACAAATTGCATTGATGAATAGCGC-3'	Specific RVS primer for RT-PCR
HA-SK3 F1	5'-GGCTGCCGCTTCCTCATCC-3'	Specific FWD primer for RT-PCR
HA-SK3 R1	5'-CGATGATGTTAACACATACATG-3'	Specific RVS primer for RT-PCR
HA-SK4 F2	5'-TGTTGCTGCCGCTTCCACT-3'	Specific FWD primer for RT-PCR
HA-SK4 R2	5'-GAAATTAAGTCGTAGTAACTACAATG-3'	Specific RVS primer for RT-PCR
HA-SK5 F1	5'-GATGAATGTGGCTGCTGCT-3'	Specific FWD primer for RT-PCR
HA-SK5 R1	5'-AGTGAAGATATATGACATCCGT-3'	Specific RVS primer for RT-PCR
HA-SK6 F1	5'-TGCTGCTGCTTCCTCATCC-3'	Specific FWD primer for RT-PCR
HA-SK6 R1	5'-CTAATGAACACATGAGCATACATC-3'	Specific RVS primer for RT-PCR
HA-SK7 F1	5'-GCGACAATGGCTCAGGCATGG-3'	Specific FWD primer for RT-PCR
HA-SK7 R1	5'-CCTTCCATATATCGTAACATGGTG-3'	Specific RVS primer for RT-PCR
HA-SK8 F1	5'-GATGAATGTGGCTGCCGCG-3'	Specific FWD primer for RT-PCR
HA-SK8 R1	5'-GGAAGAATGTCTGTTAATTTATGCAA-3'	Specific RVS primer for RT-PCR
HA-HT1 F1	5'-TCAGGCTGCTGCTTCTACC-3'	Specific FWD primer for RT-PCR
HA-HT1 R1	5'-GTAGCAAAAGGGATGGTATATCAC-3	Specific RVS primer for RT-PCR
HA-CT1 F1	5'-AATGACCACTGCTGCGTCG-3'	Specific FWD primer for RT-PCR
HA-CT1 R1	5'-TCCTTATCCTAATGGAATAATGTA-3'	Specific RVS primer for RT-PCR
HA-CT2 F1	5'-TGGAAAATGCTGGTATTCAC-3'	Specific FWD primer for RT-PCR

HA-CT2 R1	5'-CTGATTCATGGGTATTATTGTAC-3'	Specific RVS primer for RT-PCR
HA-CT3 F1	5'-TGGAAAATGCTGGTATTCAT-3'	Specific FWD primer for RT-PCR
HA-CT3 R1	5'-ACTGTACAGTGCATCATTTAC-3'	Specific RVS primer for RT-PCR
28SrRNAF870	5'-CCCGTCTTGAAACACGGACCA-3'	FWD primer for positive control
28SrRNAR1200	5'-TTCGATTAGTCTTTCGCCCCTAT-3'	RVS primer for positive control

B

Name	Sequence	Description
MHC F1	5'-CARGYNGCNYTNGARGARGC-3'	First Degeneracy FWD primer for cloning
MHC F2	5'-GYNGCNYTNGARGARGCNGA-3'	Second degeneracy FWD primer for cloning
MHC R1	5'-TCTGAAACTGACATTATAGCCCA-3'	First specific RVS primer for cloning
MHC R2	5'-CGTTTGTAAGAATGGAGGCCAAG-3'	Second specific RVS primer for cloning
HA-F1MHC F1	5'-GCATTCGTGAACTTGAAGGTC-3'	First RVS primer for 3'RACE
HA-F1MHC R1	5'-AACCTTATAGCCCATGAATCTAGG-3'	Second RVS primer for 3'RACE
HA-F2MHC F1	5'-GCCTTGAAAACTGGCAGGAAGA-3'	Specific FWD primer for RT-PCR
HA-F2MHC R1	5'-GCTCTGAAACTGACATTATAGCCC-3'	Specific RVS primer for RT-PCR
HA-S1MHC F1	5'-CGAAACCAATGCTCTGAAGAACACC-3'	Specific FWD primer for RT-PCR
HA-S1MHC R1	5'-GTAAGCATCAGTACCGTCCAAGAG-3'	Specific RVS primer for RT-PCR
HA-S2MHC F1	5'-CGCCTCGAAGAAGTTGAGGGTAAT-3'	Specific FWD primer for RT-PCR
HA-S2MHC R1	5'-ACAGGTGTTACTTGTCGGTGTCGG-3'	Specific RVS primer for RT-PCR

4-1 Expression of HA-Actins

In order to study expression pattern of twelve HA-actins, end-point RT-PCR was carried out in each tissue. First-strand cDNA was synthesized from crusher, cutter, deep abdominal flecxor, deep abdominal extensor, heart, hepatopancreas, with random haxamer and SuperscriptⅡ reverse transcriptase. PCR volume of 20 μl contain 1 μl cDNA (50ng), 2 μl 2μM each one of twelve HA-ACTIN F1, 2 μl 2μM each one of twelve HA-ACTIN R1, 0.2 μl Ex Taq polymerase, 1.6 μl dNTP (2.5mM), 2 μl 10X PCR buffer, and add the sterile water to 20 μl final volume. The PCR conditions were 94 ˚C for 1min, followed by 30 cycle of 94 ˚C for 30 s, 50˚C for 30 s, 72 ˚C for 30 s, and final elongation step at 72 ˚C for 5 min. 28s rRNA was amplified using 28s rRNA primers for positive control.

Real time PCR analysis was performed using the Chromo 4 ™ Four-Color Real-Time System (Bio-Rad, USA) to measure twelve HA-actins expression levels among different tissues (3 sample of crusher muscle, cutter muscle, deep abdominal flexor muscle, extensor muscle, heart, and hepatopancreas). SYBR Green premix Ex Taq™ (Takara. Japan) was used for real time monitoring of amplification. Real-time PCR was carried out under the same conditions as for the end-point RT-PCR described above, except that 40cycles was performed. Standard curves were constructed to quantify copy numbers as described

previously and to measure the efficiency of each primer set (Kim et al., 2005). Amplification of 28s rRNA was carried out to normalize calculated copy number from each tissue. The relative copy numbers of each tissue were calculated to following method: the actual copy numbers of HA-ACTIN/the actual copy numbers of 28S rRNA.

4-2 Expression of HA-MHCs

In order to study expression pattern of four HA-MHCs (fast muscle MHC, S1 MHC, S2 MHC and HA-F2MHC), end-point RT-PCR was carried out in each tissue. cDNA synthesised same Ha-actins method. The PCR conditions were 94 ˚C for 1min, followed by 30 cycle of 94 ˚C for 30 s, 60˚C for 20 s, 72 ˚C for 30 s, and final elongation step at 72 ˚C for 5 min. PCR content is the same as described above, but that primers used specific MHC forward and reverse primer of 4 pairs (Table 1). To measure the HA-MHCs expression level, we used real-time RT-PCR. All conditions and experimental methods were same to the strategy in HA-actins.

5. Data analysis and statistics

The deduced amino acid sequences were obtained from the web-based open reading frame (ORF) finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Multiple amino acid sequence alignment and similarity analysis were made using the ClustalW2 program which is available at European bioinformatics institute (EBI;http://www.ebi.ac.uk/Tools/clustalw2/). Analyzed data were then presented by GeneDoc program (http://www.psc.edu/biomed/genedoc). The neighbor-joining method was used for the construction of phylogenetic trees by using MEGA4 program (Tamura et al. 2007). The level of significance of actins and MHCs in each tissue were evaluated statistically by comparing the means, using Kruskal-Wallis and Wilcoxon test using SPSS (version 11.5).

RESULTS AND DISCUSSION

1. Isolation of twelve actin cDNAs and MHC from Lobster

Actins

Two series of PCR using degenerated primers and 3'RACE primers obtained specific PCR products including the crusher and cutter claw muscle, deep abdominal flecxor, deep abdominal extensor, heart and hepatopancreas tissues. To complete the full open reading frame (ORF) sequence, we amplified cDNA using degenerated primer containing start codon (ATG) and specific reverse primers within 3' UTR region. Total twelve actin cDNAs were isolated from each tissue. Each actin have common stop codon (TAA) and conserved polyadenylation signal in unique 3' UTR region. In addition, amino acid sequence of the class Ⅱ actin genes begins with Met-Cys-Asp N- terminal, whereas the actin itself begins with an acetyl (Ac)-Asp (Glu) (Sheff et al., 1992). All lobster actins show those conserved amino acid sequence. Two cDNAs from the

crusher claw muscle were named as HA-ActinSK1 and HA-ActinSK2, where HA stand for the species name '*Homarus americanus'* and SK (skeletal muscle) means the tissue type they isolated. HA-ActinSK1 is 100% identical to previously known as the actin from the American lobster (GeneBank Accession number: AF399872). Since previous nomenclature 'actin' would mislead us, we used our name, HA-ActinSK1 in this experiment. HA-ActinSK2 shared 96% nucleotide sequence identity with HA-ActinSK1 and only 3 amino acid residues were differed from HA-ActinSK1. Three HA-Actins were cloned from the cutter claw muscle tissue; HA-ActinSK3, SK4, and SK5. They showed the highest amino acid sequence identity to actin T2 from *Litopenaeus vannamei* in the GeneBank database*;* HA-ActinSK3 and SK5 shared 94% and HA-ActinSK4 shared 93%, respectively. HA-ActinSK6 was isolated from DA extensor and HA-ActinSK7 and SK8 were obtained from DA flexor muscle. HA-ActinHT1 was isolated from the heart muscle and thee actins were isolated from the hepatopancreas tissue. Since they were isolated from the non-muscle tissue and share much higher sequence similarity to cytoplasmic actin, we named them as HA-ActinCT1, CT2, and CT3 respectively. Except for HA- ActinSK2, all skeletal muscle type actins have 1134 bp nucleotide sequence encoding 377 amino acids. Whereas other actins including HA-ACTIN SK2 show lacked 3 nucletides at signal peptide sequence region (Table 2). We also amplified genomic DNA region to study genomic structure using primer set containing start codon and stop codon

sequence and all HA-ActinSKs contain no intron sequence whereas HA-ActinCT2 and CT3 have one intron (data not shown). HA-ActinCT1 failed to amplify genomic sequence. This result suggest that HA-Actins more complicated genome structure.

Table 2. Composition of actin cDNAs

Name	Total length (bp)	ORF (bp)	Accession number	Source
HA-ActinSK1	1386	1134	AF399872	Crusher
HA-ActinSK2	1395	1131	FJ217207	Crusher
HA-ActinSK3	1224	1134	FJ217208	Cutter
HA-ActinSK4	1248	1134	FJ217209	Cutter
HA-ActinSK5	1295	1134	FJ217210	Cutter
HA-ActinSK6	1243	1134	FJ217211	Extensor
HA-ActinSK7	1276	1134	FJ217212	Flexor
HA-ActinSK8	1245	1134	FJ217213	Flexor
HA-ActinHT1	1350	1131	FJ217214	Flexor
HA-ActinCT1	1258	1131	FJ217215	Hepatopancreas
HA-ActinCT2	1254	1131	FJ217216	Hepatopancreas
HA-ActinCT3	1274	1131	FJ217217	Hepatopancreas

Myosin heavy chain (MHC)

cDNA sequence of another fast type MHC was obtained by amplification using the degeneracy primer and specific primer from cutter claw muscle. We named it as HA-F2MHC for it was second MHC from fast muscle type. The cDNA sequences were 1101 bp in length with an open reading frame (ORF) of 848 bp and a 3' UTR of 253 bp with polyadenylation signal. MHC has general stop codon (TAA) (Fig. 2). The nucleotide sequence had highest sequence identity with the lobster fast and S1 MHC isoforms within the corresponding ORF (80% and 79% sequence identity to both fast and S1 MHC in the ORF). The 3' UTR of each MHC had much lower sequence similarity.

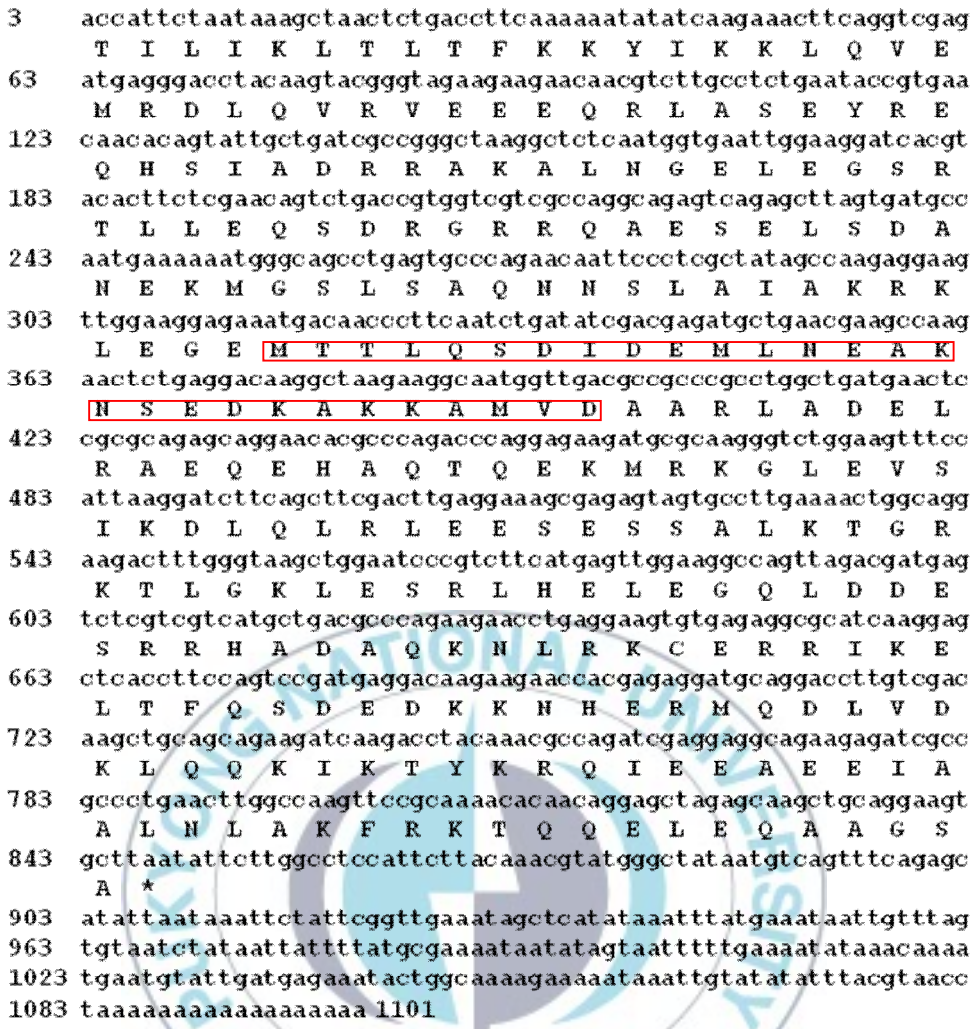


Fig. 2. The nucleotide and deduced amino acid sequences of HA-F2MHC. Box shows a repeating 28-amino acid sequence pattern. An asterisk (*) indicates the stop codon.

2. Structural analysis of lobster actins

Actin

Multiple amino acid alignment was carried out to identify and characterize structural of the deduced of HA-Actins from lobster (Fig. 3). Actins isolated from the same tissues share higher amino acid sequence similarity than ones from other tissues. First, two actins from the crusher claw muscle, HA-ActinSK1 and SK2, share 98% amino acid sequence identity whereas they share less than 90% with other actins (Table 3). Five actins from the cutter claw and deep abdominal tissues, which is composed almost of fast muscle fiber, share 95% to 98% amino acid sequence identity each other. Among actins isolated from the cutter claw muscle, HA-AntinSK3 is more similar to HA-AntinSK5 than to HA-ActinSK4. Three actins from the deep abdominal muscle, HA-ActinSK6, SK7, and SK8 share highest amino acid identity each other (Table 3). These results suggest that there is to do with structure and expression pattern in different fiber and tissue types. HA-ActinHT1, which was isolated from the heart muscle tissue, share only 90% amino acid sequence identity with HA-ActinCT1. It instead, showed 93% identity with mosquito actin (Accession no.: XM-315269) in the

NCBI database which was a proposed gene from genomic research. These results suppose that HA-ActinHT1 is unique cardiac type of actin from crustaceans. Three actins isolated from the hepatopacreas, HA-ActinCT1, 2, and 3 were also much similar each other compared to other actins. HA-ActinCT2 and HA-ActinCT3 shared 98% amino acid sequence identity while 95% with HA-ActinCT1. We also analyzed lobster actins with recently isolated two actins from Pacific white shrimp, *Litopenaeus vannamei* (Sun et al., 2007). Since it shows highest amino acid sequence identity to aHA-ActinSK3 and HA-ActinSK5 (94%), *L. vannamei* actinT2 must be the homology to actins from the cutter muscle which is mainly composed of the fast type fibers. In addition, *L. vannamei* actinT1 shared 99% amino acid sequence identity with HA-ActinCT1 suggesting that *L. vannamei* actinT1 is cytoplasmic actin not a skeletal muscular one.

Actins are conserved amino acid sequence and abundant protein more than any other protein. It consists of a single polypeptide chain of about 375 residues and contains a binding site one molecule of ATP or ADP and several divalent cations, mostly Ca^{2+} or Mg^{2+} (Kabsch et al. 1992). Actin is consised of two domains Ⅰand Ⅱ (small and large in the actin notation). Each domain subdivided into two subdomains; subdomains 2 and 4 of one molecule interact with subdomains 1 and 3 of the molecule above it (Wolfgang Kabsch et al. 1995). All twelve actins isolated from the lobster contain all those conserved characters, which suggest that these actins are functional.

We analyzed structural character of lobster actins to understand structural difference, which may distinguish physiological characters of two groups of lobster actins (Fig. 3). First, we analyzed myosin binding site if there is different in myosin binding character. Glutamic acid residue (E93), which is responsible for myosin binding, is also well conserved throughout all actins. Residue Glu^{93} has been Identified as an Amino Acid Affecting Myosin Binding (Razzaq et al., 1999). Since region for interacting with myosin is located mostly in subdomain1, amino acid residues between N92 and L104 may be responsible for interacting with different myosins. Interestingly, amino acid residues at 101 in lobster actins are either Ser or Cys whereas all mammalian actins contain His at that position. Another interesting variation within this region is at the residue 103. Val is shown in both cytosolic actins and actins from the crusher while Thr is replaced both in mammalian muscular actins and actins from cutter of lobster. Although E93 is critical for binding to myosin, variation of neighboring amino acid residues may affect of binding affinity for different types of myosin. Other conserved myosin binding sites identified by cross-linking techniques, E361, D363, and E364,(Sutoh, 1983) were well conserved except for Glu364 substitution to Asp364 in HA-ActinSK2.

Second, we also investigated the region for polymerization. Recent crystal structure study of the dimeric actin revealed a close insight of polymerization mechanism. Kudryashov et al. (Kudryashov et al., 2005) found that the

significant interactions between actin monomer occur between subdomain 4 of the lower protomer and subdomain 3 of the upper protomer. In particular, interactions between residues 197-204 in subdomain 4 of the lower protomer, and residues 287-291 and 322-326 in subdomain 3 of upper protomer are important. One salt bridge between Lys291 and Glu205 two hydrogen bonds (Lys291-S199 and T203-D288) stabilize dimerization of actin. Four additional van der Waals contacts were proposed within the contact. We analyzed corresponding amino acid residues to estimated structural difference for polymerization of actin monomers between from the crusher and the cutter claw muscles. Amino acid residues which are responsible for both salt bridge and hydrogen bonds are well conserved whereas two amino acid residues within 322-326 of subdomain 3 were different. Those two amino acid residues at 323 and 324 are involved in van der Waals contacts and difference between from crusher and cutter may explain the different counterpart for their polymerization (Fig. 3).

Finally, we compared the region for binding tropomysin & troponin complex. Milligans et al. (Milligan et al., 1987) suggested that tropomyosin runs along the interface between subdomains 3 and 4 with contacts around Lys215 and Pro307 in the presence of S1 and Ca^{2+}. Corresponding amino acid residues were conserved in all lobster actins (Fig. 3). In addition, α-helix from Asp222 to Ser233 is involved in interacting with tropomyosin and Lys238 is key residue within the

region (Szilagyi et al., 1984). Ile229 in HA-ACTINSK1 and 2 are substituted by Val229 in HA-ACTINSK3,4, and 5. This difference may influence binding affinity for each tropomyosin. Three tropomyosins were isolated from American lobster (Mykles et al., 1998) and there is a strong correlation in expression level between S1 tropomyosin and S1 MHC (Medler et al., 2004). Correlation among actins, tropomysins, and MHCs should be explained to have better insight of interacting among those fiber proteins.

Phylogenetic tree was analyzed to study evolutional relationship of twelve actins from lobster (Fig. 4). HA-ActinSKs and HA-ActinsHT1 were showed the same group with vertebrate muscular actins, whereas HA-ActinCTs were classified as cytoplasmic actins with vertebrate β-actins. HA-ActinsHT1 was grouped with HA-ActinSK branched off from vertebrate cardiac and other muscular actins. This result suggests that lobster skeletal actins were evolved from ancestral muscular actin and mammalian skeletal and cardiac muscle actins were evolved after that event. HA-ActinCT1 was group together with vertebrate beta actins whereas HA-ActinCT2 and 3 were clustered as other group suggesting these two cytoplasmic actins may be evolved as species-specific manners.

Myosin heavy chain (MHC)

We obtained partial HA-F2MHC neck the rod domain sequence. BLAST search result showed that the deduced amino acid sequence of HA-F2MHC had 77% and 79% similarity to slow muscle myosin S1 heavy chain and fast myosin heavy chain from *Homarus americanus*, respectively. In addition, the deduced amino acid sequence of HA-F2MHC exhibited high sequence similarity to various animals, including *Farfantepenaeus paulensis* (75% identity, Accession no.: AAZ20120), *Tribolium castaneum* (72% identity, Acecssion no.: XP_001813815), *Drosophila melanogaster* (71% identity, Accession no.: CAA37309), *Placopecten magellanicus* (55% identity, Accession no.: AAB03660), *Argopecten irradians* (55% identity, Accession no.: CAA39247), *Dugesia japonica* (52% identity, Accession no.: BAA34954), *Thunnus thynnus* (52% identity, Accession no.: BAA12730), *Danio rerio* (50% identity, Accession no.: NP_001108561).

The C-terminus (rod domain) encodes a α-helical secondary structure. The cloning of HA-F2MHC had a predicted α-helical secondary structure over 59.7% composed of helix at residues. The highly repetitive sequence of the striated myosin heavy chain rod region, consisting of 38 complete 28-residue repeats of heptad units, has been previously study (McLachlan and Karn, 1982, 1983). The

28-amino acid sequence contains four repeated heptapeptide result in dimerization into coiled coils via hydrophobic interactions between residues at first and fourth positions from adjacent monomers (Crick, 1953). In previous study, the lobster F1 MHC showed a repeating 28-residue pattern. Likewise, HA-F2MHC show 28-residue repeated from residue 113 to residue 132 within the corresponding F1 MHC amino sequence. Hydrophobic residues were located at position Met105, Leu108, Ile112, Met115, Ala119, Ser122 Ala126 and Ala129. The “skip” residue (McLachlan and Karn, 1982, 1983) inserted at the end of the 28-residue repeats interrupt the regular repeats, causing local instability in the coiled coil and shift in phase in the remaining repeat pattern. For case in point, there is 178 threonine (Fig. 2).

Phylogenetic analysis was carried out to understand evolutional relationship. (Fig. 4). Vertebrate and invertebrate grouped well each other. Lobster MHCs groued from invertebrate MHCs. This result suggests that lobster MHCs were evolved from ancestral MHC after any event

Table 3. Comparison of amino acid sequence of twelve actins from American lobster

	SK3	SK5	SK7	SK8	SK6	SK4	SK1	SK2	CT2	CT3	CT1	HT1
SK3	377	97%	96%	95%	96%	96%	88%	89%	84%	84%	85%	87%
	0	98%	98%	98%	98%	98%	94%	95%	93%	93%	93%	95%
	0	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SK5	366	377	97%	97%	97%	95%	89%	90%	85%	84%	86%	87%
	370	0	99%	98%	99%	97%	95%	95%	93%	93%	93%	94%
	0	0	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SK7	363	368	377	98%	98%	96%	89%	89%	85%	84%	86%	87%
	371	374	0	99%	98%	97%	94%	94%	93%	93%	93%	95%
	0	0	0	0%	0%	0%	0%	0%	0%	0%	0%	0%
SK8	361	366	371	377	98%	96%	90%	90%	85%	85%	87%	88%
	370	373	374	0	98%	98%	95%	95%	93%	93%	93%	95%
	0	0	0	0	0%	0%	0%	0%	0%	0%	0%	0%
SK6	364	367	370	370	377	96%	89%	89%	85%	85%	87%	87%
	373	374	373	372	0	97%	95%	95%	93%	93%	93%	95%
	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0%
SK4	362	361	363	363	362	377	88%	89%	84%	84%	85%	88%
	372	369	369	370	369	0	95%	95%	93%	93%	93%	95%
	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%
SK1	334	339	338	341	338	335	377	98%	88%	88%	89%	89%
	358	359	357	360	359	359	0	99%	94%	94%	94%	95%
	0	0	0	0	0	0	0	0%	0%	0%	0%	0%
SK2	336	340	339	341	339	336	373	376	89%	88%	90%	89%
	359	360	358	361	360	360	374	0	95%	94%	95%	96%
	1	1	1	1	1	1	1	0	0%	0%	0%	0%
CT2	320	323	322	324	324	320	333	335	376	98%	96%	89%
	353	354	352	353	353	353	356	358	0	99%	97%	96%
	1	1	1	1	1	1	1	0	0	0%	0%	0%
CT3	317	320	319	321	321	317	332	334	371	376	95%	89%
	352	353	351	352	352	352	355	357	373	0	97%	96%
	1	1	1	1	1	1	1	0	0	0	0%	0%
CT1	324	327	326	329	328	323	339	341	362	359	376	90%
	353	354	352	354	353	352	358	360	368	367	0	96%
	1	1	1	1	1	1	1	0	0	0	0	0%
HT1	328	328	329	334	330	332	337	338	336	335	339	376
	360	358	359	360	359	361	360	362	362	361	361	0
	1	1	1	1	1	1	1	0	0	0	0	0

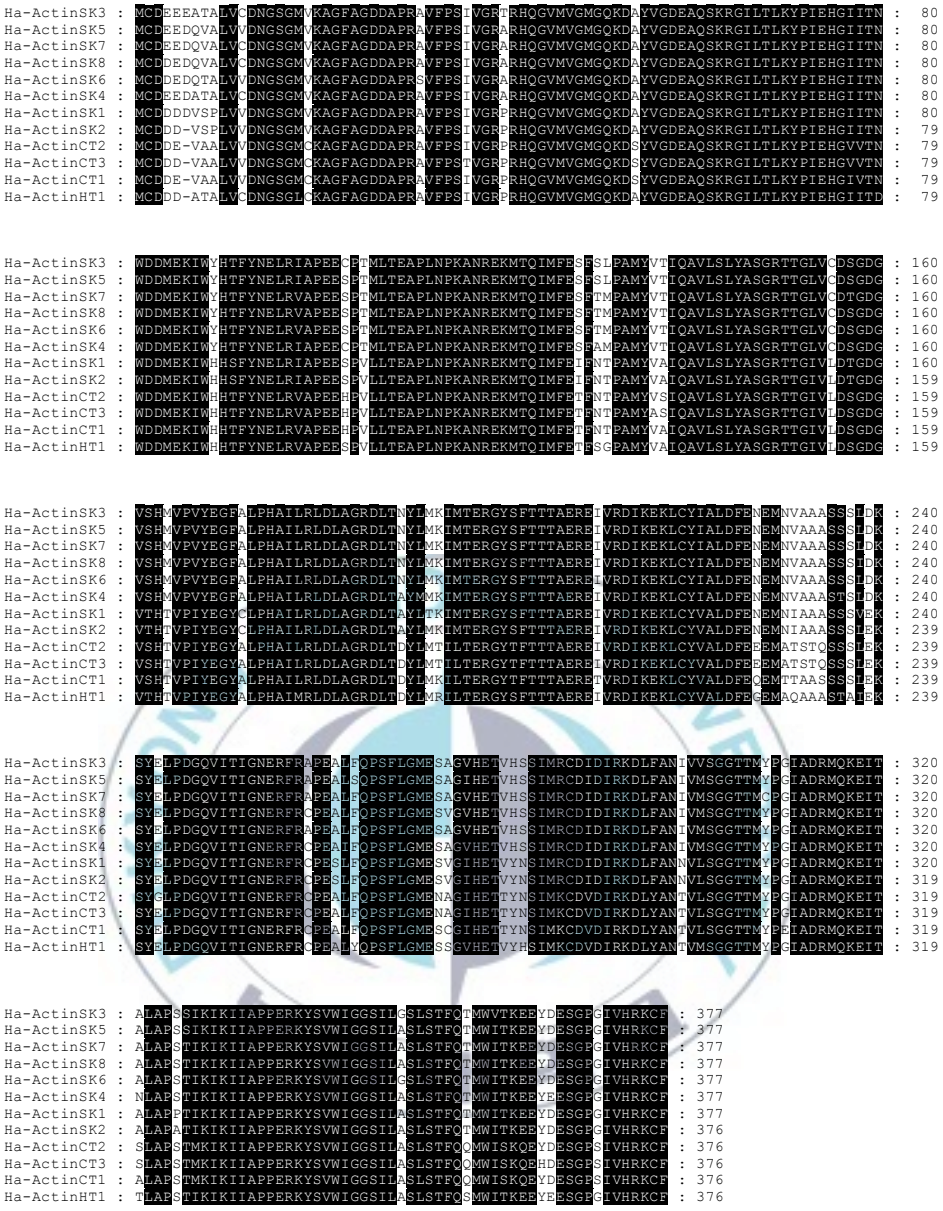
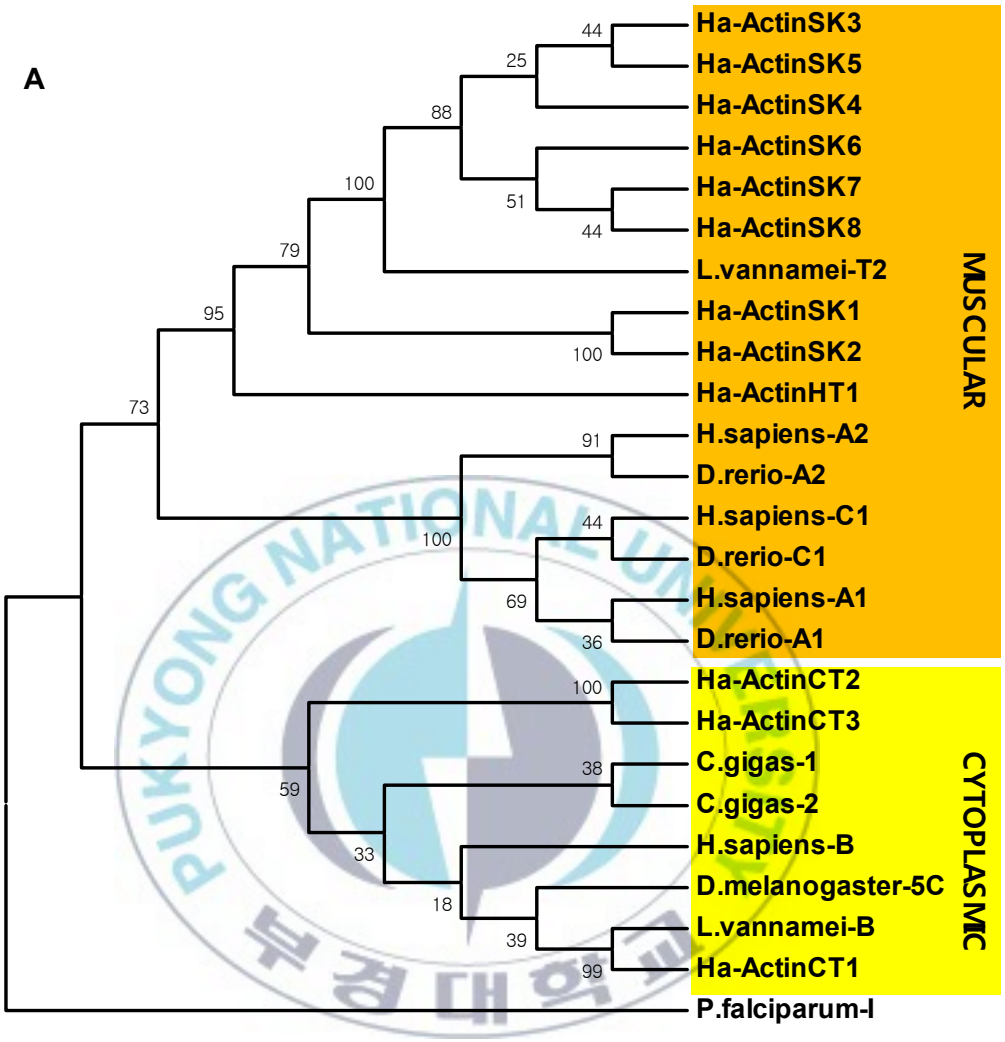


Fig. 3. Comparison of the amino acid sequences of actins from American lobster

(*Homarus americanus*). Conserved amino acid residues are shaded in black colors. The GenBank accession numbers: HA-ActinSK1 (AF399872), HA-ActinSK2 (FJ217207), HA-ActinSK3 (FJ217208), HA-ActinSK4 (FJ217209), HA-ActinSK5 (FJ217210), HA-Actin SK6(FJ217211), HA-ActinSK7 (FJ217212), HA-ActinSK8(FJ217213), HA-ActinHT1 (FJ2 17214), HA-ActinCT1 (FJ217215), HA-ActinCT2 (FJ217216), HA-ctinCT3 (FJ217217).


A
Ha-ActinSK3
Ha-ActinSK5
Ha-ActinSK4
Ha-ActinSK6
Ha-ActinSK7
Ha-ActinSK8
L.vannamei-T2
Ha-ActinSK1
Ha-ActinSK2
Ha-ActinHT1
H.sapiens-A2
D.rerio-A2
H.sapiens-C1
D.rerio-C1
H.sapiens-A1
D.rerio-A1
Ha-ActinCT2
Ha-ActinCT3
C.gigas-1
C.gigas-2
H.sapiens-B
D.melanogaster-5C
L.vannamei-B
Ha-ActinCT1
P.falciparum-I
MUSCULAR
CYTOPLASMIC
44
25
88
100
51
44
79
95
100
73
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100
69
36
100
38
59
33
18
39
99

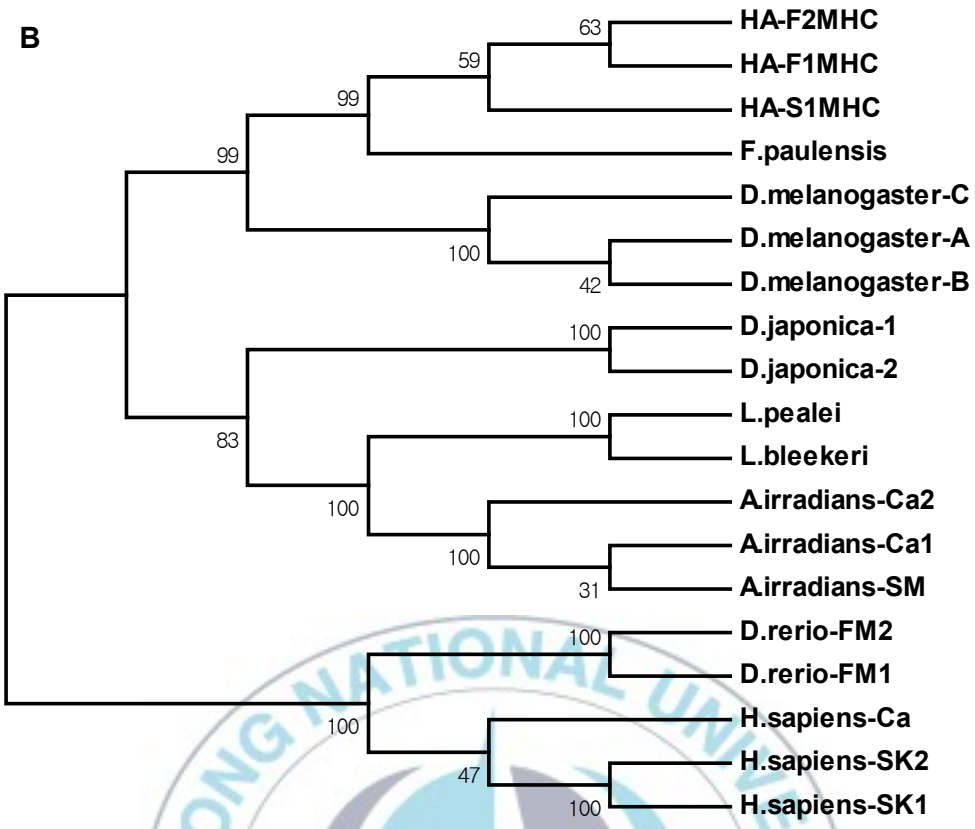


Fig. 4. Phylogenetic diagram of the different actins based on the amino acid sequence similarity. Phylogenetic tree was generated by the neighbor joining method with ClustalW progarm and represented by MEGA4 program. The Boostrapping replication was 1000. A is actins; The GenBank accession numbers: L.vannamei-T2 (AAT66425), L.vannamei-B (AAG16253), D.melanogaster-5C (NP_511052), H.sapiens-B (NP_001092), C.gigas-1 (AAB81845), C.gigas-2 (BAB84579), H.sapiens-A2 (NP_001604), D.rerio-A2 (AAH 75896), H.sapiens-C1 (NP_005150), D.rerio-C1 (NP_001002066), H.sapiens-A1(NP_00 1091), D.rerio-A1 (NP_571666), P.falciparum-I (ABO69629). The GenBank accession numbers of HA-ACTIN genes see the legend to Fig. 3. B is MHCs; The GenBank

ccession number: HA-F1MHC (AAA17371), HA-S1MHC (AAP59794), F.paulensis (AAZ20120), D.melanogaster-A (NP_724005), D.melanogaster-B (NP_724007), D.melanogaster-C (NP_723999), A.irradians-Ca2 (AAF62395), A.irradians-Ca1 (AAF62394), A.irradians-SM (AAF62392), D.japonica-1 (BAA34954), D.japonica-2 (BAA34955), D.rerio-FM2 (NP_694514), D.rerio-FM1 (AAF78476), H.sapiens-SK2 (AAI26410), H.sapiens-SK1 (NP_005954), H.sapiens-Ca (EAW66155), L.pealei (AAC24207), L.bleekeri (ACD68201).

3. Expression study

Actin

End-point RT-PCR was carried out to understand expression pattern of twelve actin genes in each tissue types (Fig. 5). All PCR products were identified as expected sizes without any nonspecific bands, which proved that all primers worked well. Both HA-ActinSK1 and SK2 showed predominant expression in the claw muscle. HA-ActinSK3 and SK4 were expressed highly in the cutter muscle and trace expression was detected weakly in the crusher claw, deep abdominal flexor and extensor. Four actins (HA-ActinsSK5, SK6, SK7, and SK8) were expressed highly in cutter muscle, deep abdominal flexor and extensor muscle which are almost composed of fast muscle fiber. These data indicate that lobster muscle is much more complicated than we expected. In addition, all skeletal actins expressed in cutter claw muscle. This result suggest that cutter claw muscle fibers are composed of various types of myofibrillar proteins. HA-ActinHT1 is expressed exclusively in the heart tissue reconfirming the idea that HA-ActinHT1 is unique crustacean cardiac actin. HA-ActinCT1 was expressed ubiquitously in each tissue but relatively higher the heart tissue than other tissue.

Both HA-ActinCT2 and CT3 were expressed strongly in the hepatopancreas. Actins of hepatocytes participate in the processes of bile formation like the transport of secretory vesicles and recovery from lesions in vertebrate model (Ishii et al., 1991; Bunton, 1995). Although it is still unknown that if these two actins are expressed in the other non-muscle tissues and what are the biological roles, both HA-ActinCT2 and CT3 may be important proteins in the lobster hepatopancreas tissue.

In order to measure the number of mRNA transcripts from twelve HA-actins, we used real-time PCR in each tissue (Table 4 and Fig. 6). HA-ActinSK1 is expressed highest in crusher muscle, which is about 4100 times more than in the deep abdominal muscle and about 74-fold higher than in the cutter muscle. Although it is also expressed predominantly in the crusher muscle, the copy number of the HA-ActinSK2 was 9 times lesser than HA-ActinSK1 in the crusher muscle and 5 times lesser than in the cutter muscle. As in crusher muscle two actins, HA-ActinSK3 and SK4 were expressed almost exclusively in the cutter muscle. In addition, HA-ActinSK4 is expressed 3 times higher than HA-ActinSK3 in the cutter muscle. HA-ActinSK5 is expressed predominant in cutter and deep abdominal muscle. However, among cutter and DA muscle were not significant mean value of expression level. As HA-ActinSK5, HA-ActinSK6 and SK7 were expressed highest in cutter and DA muscle. (Fig. 6.). Since both cutter claw and DA muscle are mainly composed of fast muscle fiber. Therefore, these three

actins may be the major component for fast muscle fibers. In addition, the copy number of HA-ActinSK7 is about 11- fold higher in the DA flexor than in the DA extensor suggesting it may plays important roles for DA flexor muscle fiber (Table 4). Finally, HA-ActinSK8 is predominantly expressed in the DA muscle, which suggests that this actin is major component of DA muscle fiber. From those results, we found that each actin is expressed in highly restricted way according to the fiber types. This may participate it possible to have unique physiological character of each skeletal muscle fiber.

We then analyzed the ratio of actins within the same tissue (Fig. 7). We found that total copy numbers of actin mRNA among different muscle tissues are similar each other suggesting isolated actins are enough to represent actins for each tissue (Table 4). Crusher muscle consisted of 90.2% of SK1 and 9.7% of SK2 actins. Since they share 98% amino acid sequence identity and crusher claw muscle reported mostly are composed of slow muscle fiber. HA-ActinSK1 and SK2 may be the slow fiber type actins and exhibit low fiber heterogenicity. On the other hand, cutter claw muscle contains about 50% of SK4, 46% of SK3 and SK5, and about 4% of five other types of actins. Although HA-ActinSK4 and SK3 are predominantly found just in the cutter claw muscle, HA-ActinSK5, SK6, SK7, SK8 are also found in DA muscle suggesting the cutter claw muscle is composed of mixture of the general fast type and specialized fast type fibers. Note that both HA-ActinSK1 and SK2 actins are also expressed in the cutter

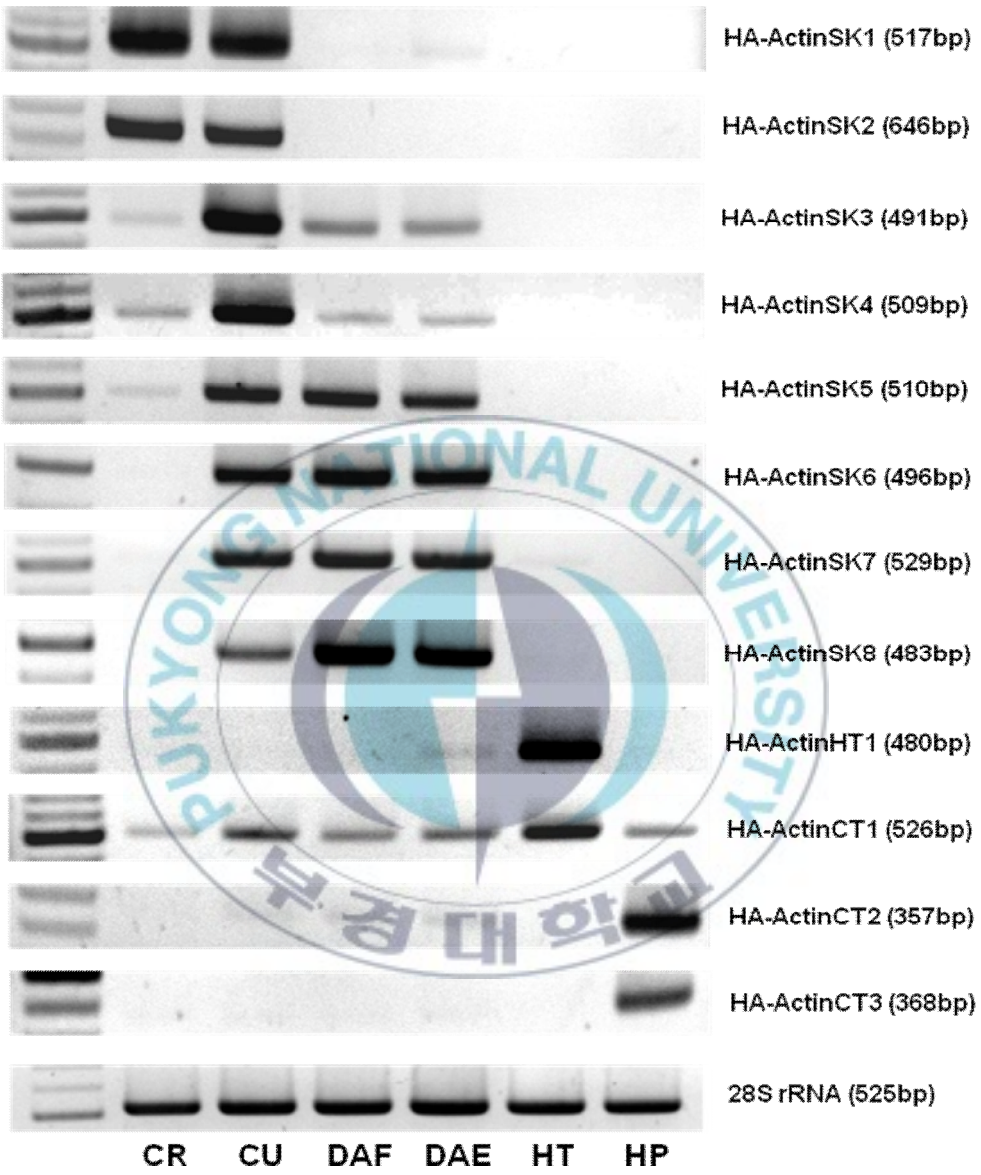
muscle exhibiting the highest heterogenecity of muscle fiber types. HA-ActinSK5 and SK8 appear to be major actins of both DA muscle types and the ratio of HA-ActinSK7 was major difference between DA flexor and DA extensor. The heart muscle was composed of exclusive HA-ActinHT1 (88.7%) and CT1(10.7%). HA-ActinCT2 (92.5%) was the major actin in hepatopancreas, and CT3 (6.2%) and CT1 (1.1%) followed.

Myosin heavy chain (MHC)

In order to study expression pattern of MHC in various tissues, we carried out End-point RT-PCR (Fig. 5). End-point RT-PCR results showed that tested MHCs were not expressed in hapatopancreas. These results suggest that hepatopancreas is not composed of muscle type MHCs and possibly other MHCs may exist. HA-F1MHC and HA-F2MHC were expressed predominantly in cutter claw muscle, deep abdominal flexor and extensor, which are mostly composed of fast muscle fiber. HA-F1MHC and HA-F2MHC was detected weakly expression in crusher claw muscle. HA-S1MHC and HA-S2MHC were expressed exclusively in claw muscle. As case of actins, these results suggest that lobster muscle is much more complicated. And cutter claw muscle fibers are composed of various types of myofibrillar.

The number of mRNA transcripts were measured using real-time PCR in each tissue (Fig. 6). The copy number of HA-F1MHC and HA-F2MHC were expressed significantly high in cutter claw muscle, deep abdominal flexor and extensor muscle. There was no statistically significant difference in copy number of HA-F1MHC and HA-F2MHC among cutter claw muscle, deep abdominal flexor, and extensor. HA-F1MHC was expressed about 3700~4200 times in the fast fiber types (cutter, DA flexor and DA extensor) than in the crusher claw muscle (Table 4). HA-F2MHC was expressed about 2300~2500 times in the fast fiber types (cutter, DAF and DAE) than crusher claw muscle. These results suggest that HA-F1MHC and HA-F2MHC are fast muscle-specific type. HA-S1MHC and HA-S2MHC are expressed exclusively in claw muscles. HA-S1MHC and HA-S2MHC were expressed about 22 and 2 times higher in crusher claw muscle than in cutter claw muscle. These results are similar to previously study (Medler et al., 2003, 2004).

A



B

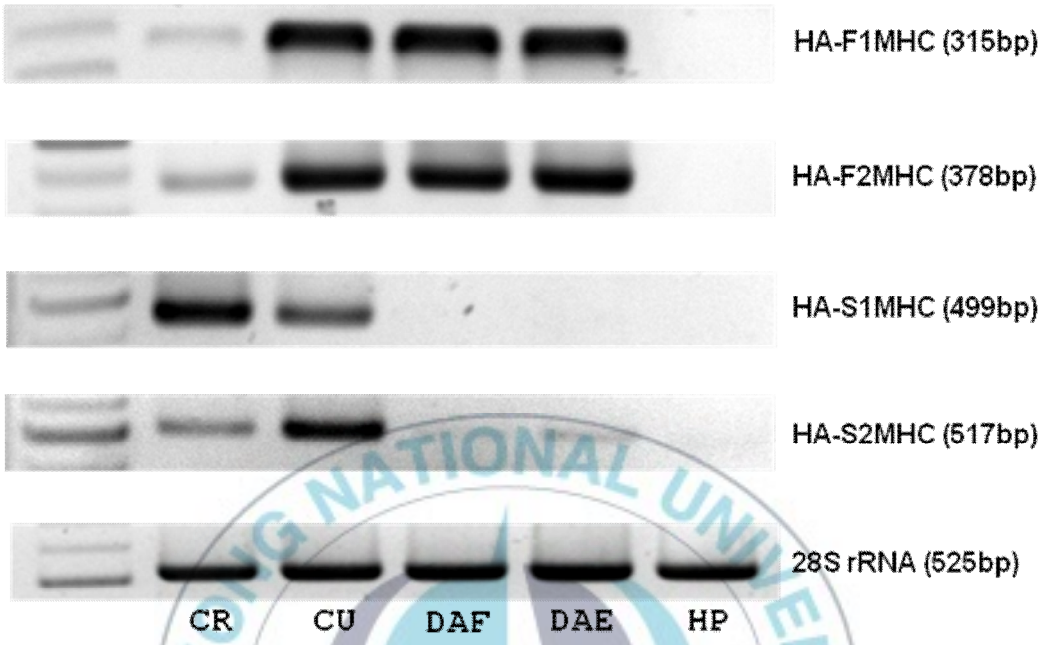
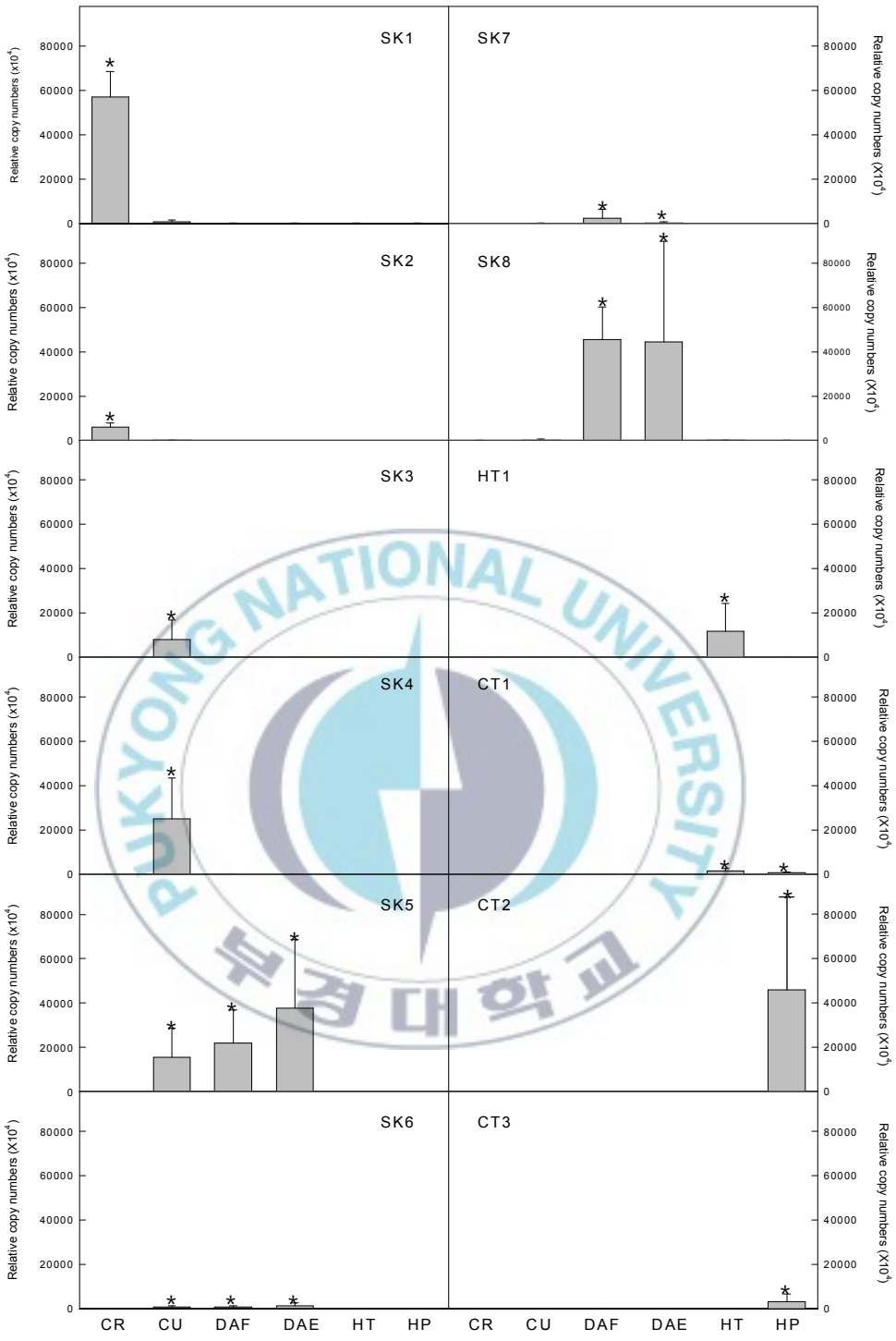


Fig. 5. Expression of HA-ACTIN (A) and HA-MHC (B) genes in various tissues. Regular end-point RT-PCR was carried out and amplicons were run on 1.5% agarose gel for 20 min. The 28S rRNA gene was used as a positive control. CR, crusher claw muscle; CU, cutter claw muscle; DAF, deep abdominal flexor; DAE, deep abdominal extensor; HT, heart; HP, hepatopancreas

A



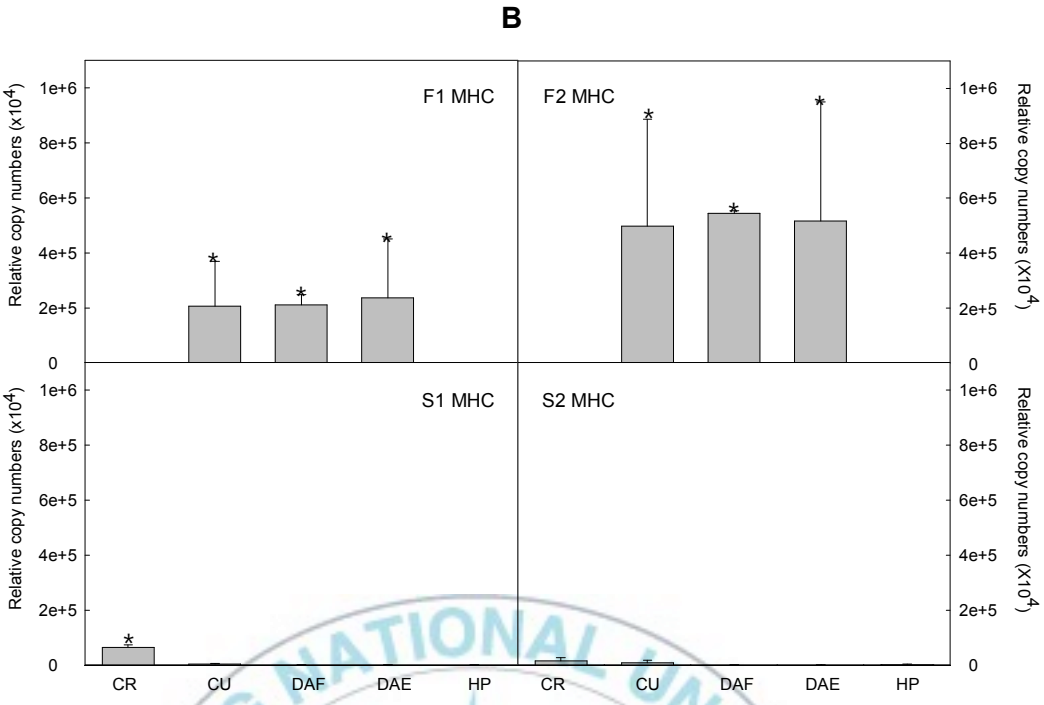
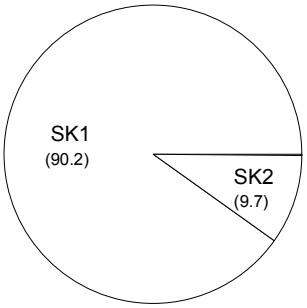
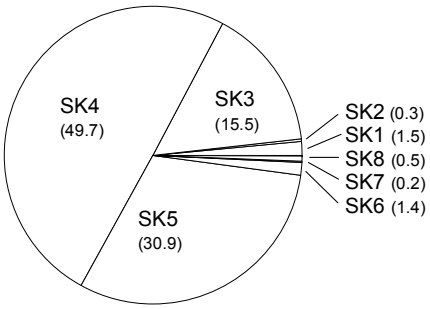


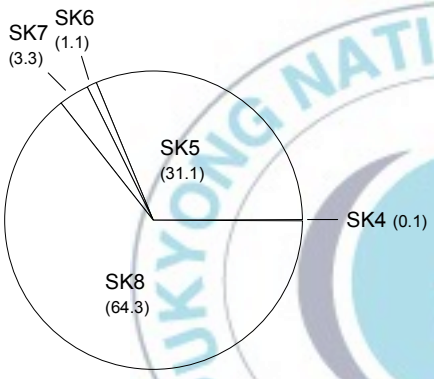
Fig. 6. Relative copy numbers of Ha-actins (A) and HA-MHCs (B) in each tissue of *H. americanus.* Copy numbers were normalized by the number of 28S rRNA. Single and double asterisk indicate the statistical significance (P <0.05) of relative copy number among different tissues. Values of y axis was showed as multiplying relative copy numbers by 10^4 for recognizing differences of relative copy numbers among tissues easily. CR, crusher muscle; CU, cutter muscle; DAF, deep abdominal flexor muscle; DAE, deep abdominal extensor; HT, heart; HP, hepatopancreas.



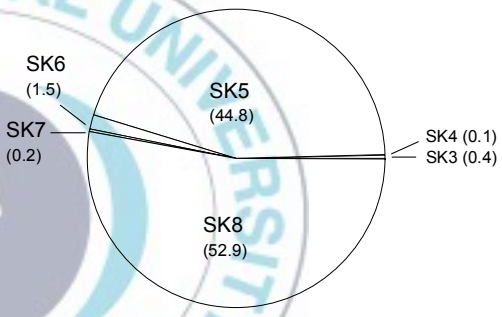
CR



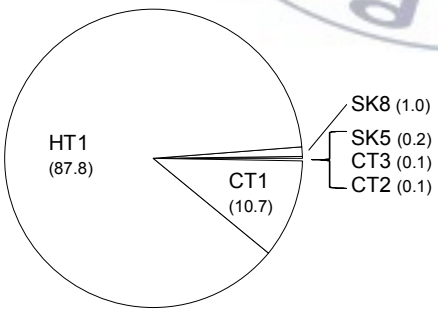
CU



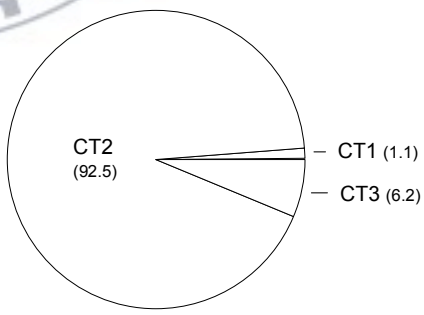
DAF



DAE



HT



HP

Fig. 7. Component ratio of HA-Actins within same tissue throughout relative copy numbers of HA-Actins in *H. americanus.* CR, crusher muscle; CU, cutter muscle; DAF, deep abdominal flexor muscle; DAE, deep abdominal extensor; HT, heart; HP, hepatopancreas.

Table 4. Relative copy number of twelve actin (A) and (B) mRNAs. CR: crusher claw muscle, CU: cutter claw muscle, DAF: deep abdominal flexor muscle, DAE: deep abdominal extensor muscle, HT: heart muscle. HP: hepatopancreas

A

	CR	CU	DAF	DAE	HT	HP
SK1	55524.6	148.1	10.9	0.7	0.9	0.0
SK2	12099.4	0.0	0.0	0.0	0.0	0.0
SK3	3.3	2530.2	19.0	6.3	3.5	3.0
SK4	3.9	11910.7	101.8	17.7	5.1	4.7
SK5	0.0	6230.4	3321.2	6929.6	0.0	0.0
SK6	3.2	956.3	445.6	478.3	1.1	33.4
SK7	4.1	596.1	993.9	1462.7	5.4	5.7
SK8	8.9	896.5	38695.4	12612.8	35.0	20.8
HT1	1.9	2.3	5.9	1.1	3301.1	14.5
CT1	8.4	6.1	7.3	5.9	101.4	93.8
CT2	2.3	4.7	6.6	3.3	2.9	6018.8
CT3	3.0	1.4	3.2	1.9	2.8	424.4
Total	40832.6	34544.6	55113.0	67805.1	12558.7	51225.1

B

	CR	CU	DAF	DAE	HP
F1MHC	50.7	187610.6	190997.4	214887.2	77.6
F2MHC	213.9	499497.2	544370.1	517698.8	23.4
S1MHC	62991.1	2803.9	8.7	17.8	8.3
S2MHC	14854.2	7452.6	121.9	360.7	1631.5
Total	78109.9	697364.3	735498.1	732964.5	1740.8

Twelve actin and MHC sequences isolated in this study present more questions. First, we need to find the correlationship in expression between twelve actins and other fiber protein components including myosin heavy chains (MHC), tropomyosins, troponins. Since different muscle fiber types are assembled by different myofibril proteins (Mykles 1985b), result of this study will help to understand the spontaneous assemblages of different fiber proteins according to the fiber types. Second, it is also needed to know which types of actins are involved in muscle plasticity including molt-induced muscular atrophy or fiber type switching in the lobster claws. During atrophy there is a preferential loss of thin myofilaments which is composed of actin and tropomyosin fibers (Mykles et al. 1981). Which actin gene is regulated by molting stages out of these actins and, if it is true, what kind of signaling pathways affect those regulations is still unclear. Muscle fiber is known to be affected by steroid

hormone in mammal and recent study reported that 20-Hydroxyecdysone increases fiber size in a muscle-specific fashion in rat (Toth et al., 2008). Since muscle plasticity is strongly related to the signaling pathway induced by ecdysone in crustacean, study of relationship between actin and ecdysone would help to understand crustacean muscle dynamics. In summary, we isolated twelve actin and MHC cDNA, and found that each actin and MHC showed unique structural and expressional characters. The actin and MHC associated myofibril proteins will be good model system to understand molecular mechanism of muscle plasticity.

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