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Twelve actin-encoding cDNAs from the American lobster, *Homarus americanus*: Cloning and tissue expression of eight skeletal muscle, one heart, and three cytoplasmic isoforms

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ABSTRACT

Lobster muscles express a diverse array of myofibrillar protein isoforms. Three fiber types (fast, slow-twitch or S1, and slow-tonic or S2) differ qualitatively and quantitatively in myosin heavy and light chains, troponin-T, -I, and -C, paramyosin, and tropomyosin variants. However, little is known about the diversity of actin isoforms present in crustacean tissues. In this report we characterized cDNAs that encode twelve actin isoforms in the American lobster, *Homarus americanus*: eight from skeletal muscle (Ha-ActinSK1-8), one from heart (Ha-ActinHT1), and three cytoplasmic type actins from hepatopancreas (Ha-ActinCT1-3). All twelve cDNAs were products of distinct genes, as indicated by differences in the 3′-untranslated regions (UTRs). The open reading frames specified polypeptides 376 or 377 amino acids in length. Although key amino residues are conserved in the lobster actins, variations in nearby sequences may affect actin polymerization and/or interactions with other myofibrillar proteins. Quantitative reverse transcription-polymerase chain reaction showed muscle fiber type- and tissue-specific expression patterns. Ha-Actin-HT1 was expressed exclusively in heart (87% of the total; 12% of the total was Ha-ActinCT1). Ha-ActinCT1 was expressed in all tissues, while CT2 and CT3 were expressed only in hepatopancreas, with Ha-ActinCT2 as the major isoform (93% of the total). Ha-ActinSK1 and SK2 were the major isoforms (88% and 12% of the total, respectively) in the S1 fibers of crusher claw closer muscle. Fast fibers in the cutter claw closer and deep abdominal muscles differed in SK isoforms. Ha-ActinSK3, SK4, and SK5 were the major isoforms in cutter claw closer muscle (12%, 48%, and 37% of the total, respectively). Ha-ActinSK5 and SK8 were the major isoforms in deep abdominal flexor (31% and 65% of the total, respectively) and extensor (46% and 53% of the total, respectively) muscles, with SK6 and SK7 expressed at low levels. These data indicate that fast fibers in cutter claw and abdominal muscles show a phenotypic plasticity with respect to the expression of actin isoforms and may constitute discrete subtypes that differ in contractile properties.

1. Introduction

American lobster has been used as model system for the study of skeletal muscle plasticity, as the claw closer muscles undergo dramatic changes in fiber phenotype and mass during development and growth. During premolt, the closer muscle atrophies to facilitate withdrawal of the claw at ecdysis (Mykles, 1997; Mykles and Skinner, 1990). During juvenile development, fiber transformation occurs as claws differentiate into the asymmetric claws of the adult. Fast fibers switch to slow fibers in the presumptive crusher claw, while slow fibers switch to fast fibers in the presumptive cutter claw (Govind, 1992; Govind et al., 1987; Medler et al., 2007; Mykles, 1997).

Three muscle fiber types are recognized in crustacean species: fast (F), slow-twitch (S1), and slow-tonic (S2). The types differ qualitatively and quantitatively in the expression of myofibrillar protein isoforms (Li and Mykles, 1990; Mykles, 1985a,b, 1988; Mykles et al., 2002; Silverman et al., 1987). In lobster, fast fibers are located in the central and dorsal regions of the cutter claw closer muscle and in the deep abdominal flexor and extensor muscles; S1 fibers are located in the crusher claw closer muscle, the ventral region of the cutter claw closer muscle, the central region of claw and leg opener muscle, and the superficial abdominal flexor and extensor muscles; and S2 fibers are located in the

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Table 1
PCR primers for tissue expression of lobster actin isoforms using endpoint and quantitative RT-PCR (see Materials and methods section).

Primer	Sequence
SK1 FP1	5′-CATTGCTGCTGCCTCTTCT-3′
SK1 RP1	5′-TGCTGTTGTGACTCATTATCT-3′
SK2 FP1	5′-GATGAACATTGCTGCTGCA-3′
SK2 RP1	5′-ACAAATTGCATTGATGAATAGCGC-3′
SK3 FP1	5′-GGCTGCCGCTTCCTCATCC-3′
SK3 RP1	5′-CGATGATGTTAACACATACATG-3′
SK4 FP2	5′-TGTTGCTGCCGCTTCCACT-3′
SK4 RP2	5′-GAAATTAAGTCGTAGTAACTACAATG-3′
SK5 FP1	5′-GATGAATGTGGCTGCTGCT-3′
SK5 RP1	5′-AGTGAAGATATATGACATCCGT-3′
SK6 FP1	5′-TGCTGCTGCTTCCTCATCC-3′
SK6 RP1	5′-CTAATGAACACATGAGCATACATC-3′
SK7 FP1	5′-GCGACAATGGCTCAGGCATGG-3′
SK7 RP1	5′-CCTTCCATATATCGTAACATGGTG-3′
SK8 FP1	5′-GATGAATGTGGCTGCCGCG-3′
SK8 RP1	5′-GGAAGAATGTCTGTTAATTTATGCAA-3′
HT1 FP1	5′-TCAGGCTGCTGCTTCTACC-3′
HT1 RP1	5′-GTAGCAAAAGGGATGGTATATCAC-3
CT1 FP1	5′-AATGACCACTGCTGCGTCG-3′
CT1 RPP1	5′-TCCTTATCCTAATGGAATAATGTA-3′
CT2 FP1	5′-TGGAAAATGCTGGTATTCAC-3′
CT2 RP1	5′-CTGATTCATGGGTATTATTGTAC-3′
CT3 FP1	5′-TGGAAAATGCTGGTATTCAT-3′
CT3 RP1	5′-ACTGTACAGTGCATCATTTAC-3′
28S rRNA FP	5′-CCCGTCTTGAAACACGGACCA-3′
28S rRNA RP	5′-TTCGATTAGTCTTTCGCCCCTAT-3′

Abbreviations: CT, cytoplasmic type; FP, forward primer; HT, heart; RP, reverse primer; and SK, skeletal muscle.

distal region of the cutter claw closer, the proximal and distal regions of the claw and leg opener, and superficial abdominal flexors and extensor muscles (Govind et al., 1987; Medler and Mykles, 2003; Mykles 1985a,b, 1988; Mykles et al., 2002).

In order to study phenotypic plasticity in lobsters, cDNAs encoding actin (designated Ha-ActinSK1 in this study), myosin heavy chain (F, S1, and S2 isoforms), tropomyosin (F, S1, and S2 isoforms), and P75 have been cloned (Cotton and Mykles, 1993; Koenders et al., 2002; Medler and Mykles, 2003; Medler et al., 2004; Mykles et al., 1998). Recent studies indicate that crustaceans show a greater fiber phenotypic heterogeneity that blurs the distinction between S1 and S2 fibers. Slow fibers in the superficial abdominal and leg opener, and claw opener and closer muscles display a continuum in expression of myosin heavy chain, tropomyosin, and troponin isoforms, with the S1 and S2 fibers representing extremes in the range of expression patterns (Medler and Mykles, 2003; Medler et al., 2004). Expression of S1 and S2 isoforms is correlated with the synaptic properties of motor axons innervating the fibers; the S2 phenotype is associated with synapses that show greater short-term facilitation (Mykles et al., 2002). Moreover, there is increasing evidence that fast fibers are not homogeneous. In freshwater yabby, fast fibers in the claw closer and deep abdominal muscles consist of discrete subtypes, based on sarcomere lengths, Ca^{2+} activation properties, and troponin-I isoforms (Koenders et al., 2004). In lobster, fast fibers in the cutter closer and deep abdominal muscles differ in the expression of myosin heavy chain (F and S1 isoforms) and Ha-ActinSK1 (Medler and Mykles, 2003; Medler et al., 2005).

Actins comprise a multigene family with diverse genomic organization and expression patterns depending on species, tissue, and developmental stage (Hooper and Thuma, 2005). Among crustaceans, 8–10 genes are estimated in *Artemia* and 7–8 genes in *Gecarcinus lateralis* (Macias and Sastre, 1990; Ortega et al., 1992; Varadaraj et al., 1996). The number and types of actin genes in lobster are not known. Only a single actin cDNA has been characterized (Koenders et al., 2002), but expression analysis suggests that there are multiple isoforms in skeletal muscle (Medler and Mykles, 2003; Medler et al., 2005). The mRNA levels of Ha-ActinSK1, S1 tropomyosin, and S1 myosin heavy chain are highly correlated, suggesting that there are actin isoform counterparts to the F and S2 tropomyosin and myosin heavy chain isoforms (Medler and Mykles, 2003; Medler et al., 2005). Consequently, we used a modified PCR procedure on polyadenylated RNA from multiple tissues in an attempt to clone all the actins expressed in *H. americanus*. Twelve actin cDNAs, including the actin characterized by Koenders et al. (2002), were obtained. Expression of all 12 isoforms in heart, skeletal muscles, and hepatopancreas was analyzed using endpoint and real-time RT-PCR. The results indicate an unprecedented diversity of actin isoforms expressed in crustacean tissues.

2. Materials and methods

2.1. Experimental animals

Adult lobsters (*Homarus americanus*) were purchased from a local seafood market and held for at least one week in circulating aerated seawater (6 °C) on a 12-h day and 12-h night cycle. They were fed raw shrimp daily.

2.2. Cloning of Ha-Actins

RNA from lobster tissues was purified using the Trizol reagent according to the manufacturer's instructions (Invitrogen, USA) and quantified with a ND-1000 NanoDrop UV spectrophotometer (Nanodrop Technologies, Inc.). A modified 3′ RACE was used to obtain cDNA sequences containing the 3′UTR region. cDNA was synthesized using a reverse primer (5′-CTGTGAATGCTGCGACTACGATTTTTTTTTTTTTTT-3′). The reaction (12 μL) containing 3 μg total RNA, 1 μL 20 μM 3′ RACE primer and 4 μL dNTPs (2.5 mM) was heated to 70 °C for 5 min and chilled on ice for 2 min. First-strand buffer (5×, 4 μL), 2 μL 0.1 M DTT and 1 μL RNase out were added to the reaction mixture, which was incubated at 42 °C for 2 min. Superscript™ reverse transcriptase (1 μL) was added and then the mixture was incubated at 42 °C for 50 min. PCR was carried out with nested degenerate forward primers (5′-TCYGGYGGYACCAC-CATGAC-3′ first-round primer and 5′-GGYGGYACCACCATGACCC-3′ second-round primer) and sequence-specific reverse primers (5′-TGT-GAATGCTGCGACTAC-3′ first-round primer and 5′-TGAATGCTGCGAC-TACGA-3′ second-round primer). Reactions (30 μL) contained cDNA (100 ng), primers (1 μL 100 μM), Takara Ex Taq polymerase (0.2 μL), dNTP (2.5 mM), and 3 μL 10× buffer (Takara Bio Inc., Japan). PCR conditions were 1 min at 94 °C; 40 cycles at 94 °C for 1 min, 50 °C for 30 s, and 72 °C for 30 s; and post-extension at 72 °C for 5 min. PCR products were separated with 1.5% agarose gel electrophoresis and stained with ethidium bromide. PCR products were isolated from gel slices using Gel Extraction Kit (Bioneer Inc., Korea), ligated into TA plasmid vector with the pGEM-T Easy Cloning Kit (Promega, USA), and transformed into

Table 2
Properties of cDNAs encoding twelve actin isoforms in American lobster 2.1.

Name	Total length (bp)	ORF (bp)	Accession number	Tissue source
Ha-ActinSK1	1386	1134	AF399872	Crusher claw (S1 fibers)
Ha-ActinSK2	1395	1131	FJ217207	Crusher claw (S1 fibers)
Ha-ActinSK3	1224	1134	FJ217208	Cutter claw (fast)
Ha-ActinSK4	1248	1134	FJ217209	Cutter claw (fast)
Ha-ActinSK5	1295	1134	FJ217210	Cutter claw (fast)
Ha-ActinSK6	1243	1134	FJ217211	Deep abdominal extensor (fast)
Ha-ActinSK7	1276	1134	FJ217212	Deep abdominal flexor (fast)
Ha-ActinSK8	1245	1134	FJ217213	Deep abdominal flexor (fast)
Ha-ActinHT1	1350	1131	FJ217214	Heart
Ha-ActinCT1	1258	1131	FJ217215	Hepatopancreas
Ha-ActinCT2	1254	1131	FJ217216	Hepatopancreas
Ha-ActinCT3	1274	1131	FJ217217	Hepatopancreas

Ha-ActinSK1 was identical to a cDNA sequence reported by Koenders et al. (2002). ORF, open reading frame.

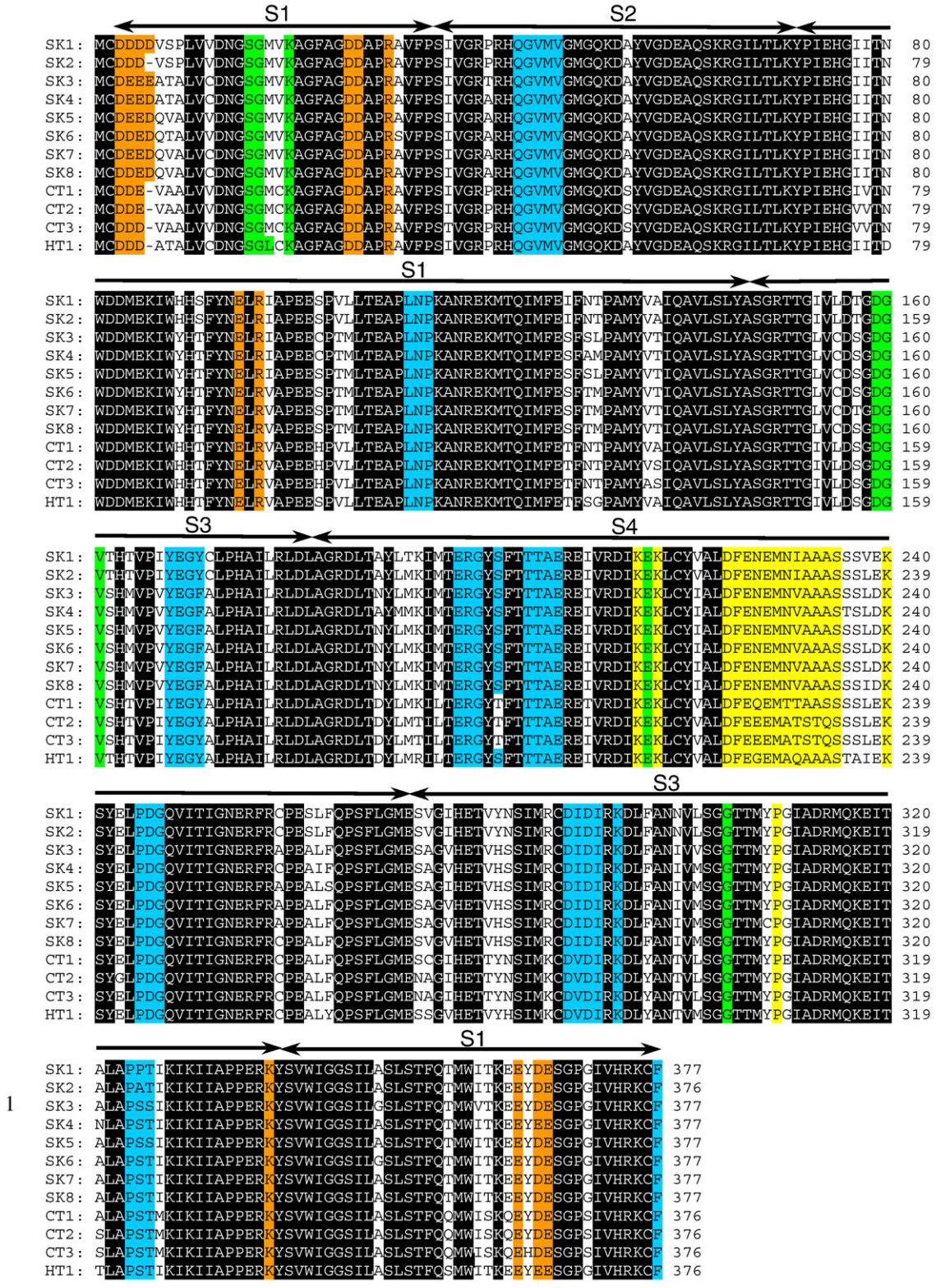

Fig. 1. Alignment of deduced amino acid sequences of 12 actin cDNAs from American lobster (*Homarus americanus*). Polypeptides were either 376 or 377 amino acids in length; the difference was due to the presence of 3 or 4 acidic residues near the N-terminus (positions #4–6). The sequence is organized into 4 subdomains (S1, S2, S3, and S4). Acidic and basic residues involved in myosin binding are shaded in orange; residues involved in ATP binding are shaded in green; regions involved in actin binding are shaded in blue; and residues involved in tropomyosin binding during the on-state (in the presence of Ca^{2+}) are shaded in yellow. Other amino acid residues identical in all 12 sequences are shaded in black. GenBank accession numbers are given in Table 2.

XL1-blue competent cells. cDNAs were sequenced with an automated DNA sequencer (ABI Biosystem, USA). The remainder of the ORF was obtained using RT-PCR with a degenerate forward primer (5′-ATGTGY-GAYGANGANG-3′) directed to the first six amino acids (M-C-D-D/E-D/E-D/E/V/A), in combination with sequence-specific reverse primer for each actin 3′-UTR (Table 1). The PCR conditions were the same as above.

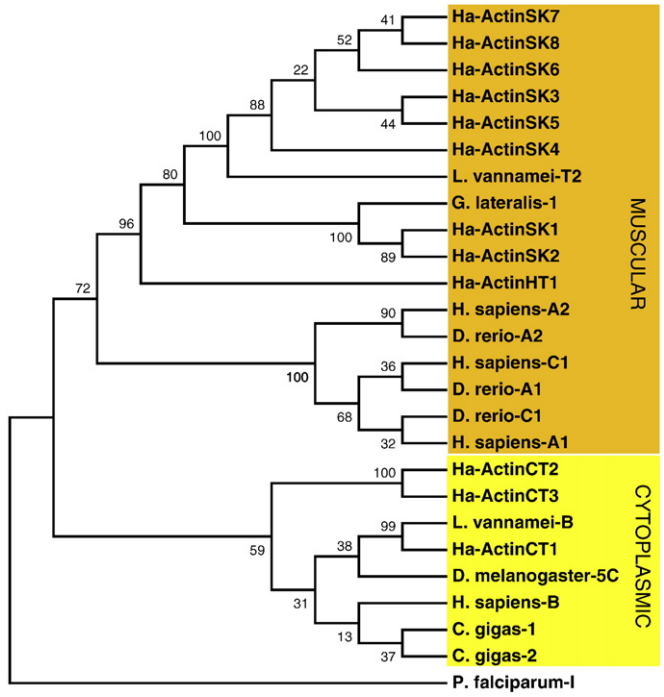

Fig. 2. Phylogenetic tree comparing the deduced amino acid sequences of lobster actins with crab, shrimp, zebrafish, oyster, and human actins. The tree was generated using the neighbor joining method (MEGA4 software) with bootstrapping replication set at 1000. A protozoan actin (*Plasmodium falciparum*-I (ABO69629) was used to root the tree. Species and GenBank accession numbers: *Litopenaeus vannamei*-T2 (AAT66425), *L. vannamei*-B (AAG16253), *G. lateralis*-1 (L76943), *Drosophila melanogaster*-5C (NP511052), *Homo sapiens*-B (NP001092), *H. sapiens*-A2 (NP001604), *H. sapiens*-A1 (NP001091), *H. sapiens*-C1 (NP005150), *C. gigas*-1 (AAB81845), *C. gigas*-2 (BAB84579), *Danio rerio*-A2 (AAH75896), *D. rerio*-C1 (NP001002066), and *D. rerio*-A1 (NP571666). GenBank accession numbers for lobster actins are given in Table 2.

Complete sequences were obtained with BLAST 2 SEQUENCES software (http://blast.ncbi.nlm.nih.gov/bl2seq/wblast2.cgi) and confirmed with PCR using specific forward and reverse primers directed to the 5′ and 3′ ends of each sequence.

2.3. Analysis of deduced amino acids sequences

Deduced amino acid sequences were obtained using an ORF finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Multiple amino acid sequence alignment and similarity analysis used the ClustalW2 program available from the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/clustalw2/). A phylogenetic tree was constructed using the MEGA4 program (Tamura et al., 2007).

2.4. Tissue expression of lobster actins

A qualitative tissue expression profile of the 12 actin isoforms was obtained using endpoint RT-PCR. cDNA was synthesized as described above, except random hexamer primers were used for reverse transcription. cDNA was treated with DNase I to remove genomic DNA (Promega. USA). The PCR mixture (20 μL) contained 1 μL cDNA (100 ng), 2 μL 2 μM forward primer (Table 1), 2 μL 2 μM reverse primer (Table 1), 0.2 μL Takara Ex Taq polymerase, 2.5 mM dNTP, and 2 μL 10× buffer (Takara Bio Inc., Japan). PCR using primers to the 28 S rRNA served as a positive control (Table 1). PCR conditions were 1 min at 94 °C, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. PCR products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide.

Real-time PCR was carried out using the Chromo 4™ Four-Color Real-Time System (Bio-Rad, USA) to quantify actin isoform mRNA levels among six tissues. The medial muscles fiber bundles from each tissue were taken for measuring actin transcripts. SYBR Green premix Ex TaqTM (Takara Bio Inc., Japan) was used with 50 ng cDNA as template. PCR used the same conditions as those for the end-point RT-PCR described above, except that 40 cycles was used. Standard curves were constructed to quantify copy numbers and to measure the efficiency of each primer set (Kim et al., 2005). Calculated copy number from each tissue was normalized to the copy number of 28S rRNA. One-way of analysis variance followed by LSD multiple comparisons used SPSS software (version 11.5). The results were considered significant at $P<0.05$.

3. Results and discussion

3.1. Isolation of twelve actin cDNAs from American lobster

Partial cDNAs encoding actins were obtained using a modified PCR with two nested degenerate forward primers to conserved amino acid sequences in actins and two nested 3′-RACE primers. cDNAs from cutter and crusher claw closer muscle, deep abdominal muscle, heart, and hepatopancreas poly(A) RNA were sequenced. Each PCR product contained the 3′ end of the highly conserved open reading frame (ORF) and a unique 3′-UTR. Since subsequent PCRs used a degenerate forward primer directed to the first six codons in the ORF of class II actin genes (Sheff and Rubenstein, 1992), none of the cDNAs have a 5′-UTR sequence. A total 12 actin cDNAs were isolated (Table 2). Each actin had a common stop codon (TAA) and polyadenylation signal. Two cDNAs cloned from the crusher claw muscle were designated Ha-ActinSK1 and Ha-ActinSK2 (*Homarus americanus* actin skeletal muscle 1 and 2). Ha-ActinSK1 was 100% identical to a cDNA characterized by Koenders et al. (2002; GenBank AF399872). Ha-ActinSK1 and SK2 shared 96% nucleotide sequence identity. Three cDNAs cloned from the cutter claw muscle tissue (Ha-ActinSK3, SK4, and SK5) showed the highest identity to actin-T2 from *Litopenaeus vannamei* (Fig. 2). Ha-ActinSK3 shared 93% nucleotide identity with SK4 and 94% identity with SK5. Three cDNAs (Ha-ActinSK6, SK7, and SK8) were cloned from deep abdominal muscles (Table 2). Seven of the 8 skeletal muscle actin cDNAs had a 1134-bp ORF encoding a 377-amino acid protein; the only exception was Ha-ActinSK2, which had a 1131-bp ORF (Table 2; Fig. 1). One cDNA encoding a 376-amino acid protein was cloned from heart (Ha-ActinHT1; Table 2). Three cDNAs, also encoding 376-amino acid proteins, were cloned from hepatopancreas (Table 2). Since they were isolated from a non-muscle tissue and shared higher sequence similarities to cytoplasmic actins (Fig. 2), they were designated Ha-ActinCT1, CT2, and CT3 (Table 2). Genomic DNA was amplified using a primer set flanking the entire ORF. All Ha-ActinSKs lacked an intron, whereas Ha-ActinCT2 and CT3 had an intron within the ORF (data not shown). We failed to amplify the Ha-ActinCT1 genomic sequence, which may indicate a more complex gene structure.

3.2. Structural analysis of lobster actins

Multiple amino acid alignments were used to compare the structural features of the 12 actins (Fig. 1). In general, actins isolated from the same tissue shared higher amino acid sequence identities than with actins from other tissues. This was apparent at two “variable regions”, a 5–6 amino acid sequence at the N-terminus (#4–8 or #4–9) and a 10 amino acid sequence at #229–238 or #230–239 (Fig. 1). The two “slow-type” actins from crusher claw muscle, HA-ActinSK1 and SK2, shared 98% amino acid sequence identity, whereas they shared 88% to 90% identity with other actins (Figs. 1 and 2). The six actins from fast muscles (cutter claw closer and deep abdominal flexor and extensor) shared 95% to 98% amino acid sequence identity. Among actins from the cutter claw muscle, Ha-ActinSK3 was more similar to SK5 than to SK4 (differences in 14 and 11 amino acids between SK3 and SK4 between SK3 and SK5, respectively; Fig. 1). Three actins from the deep abdominal muscles, Ha-ActinSK6, SK7, and SK8, shared ~98% amino acid identity (differences in

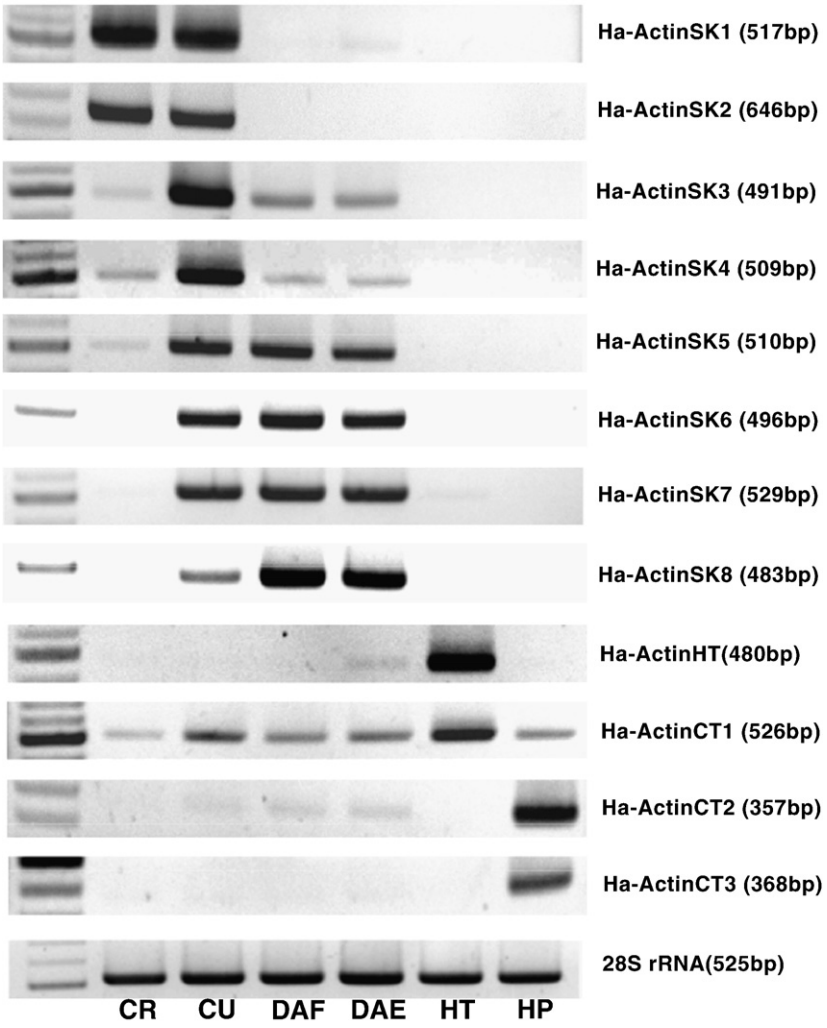

Fig. 3. Tissue expression of lobster actin isoforms using endpoint RT-PCR. Products were separated on a 1.5% agarose gel and stained with ethidum bromide (reverse image). The 28S rRNA was used as a positive control. Ha-ActinSK1 and SK2 were expressed in S1 fibers of crusher claw closer muscle (CR); SK3-8 were expressed in fast fibers of cutter claw closer muscle (CU), deep abdominal flexor muscle (DAF), and deep abdominal extensor muscle (DAE); Ha-ActinHT1 was expressed in heart (HT); Ha-ActinCT1 in all tissues; and Ha-ActinCT2 and CT3 in hepatopancreas (HP). DNA size marker on left.

7 amino acids between SK6 and SK7 and in 6 amino acids between SK6 and SK8; Fig. 1). Ha-ActinHT1 appears to be a "cardiac type" actin, as it was expressed exclusively in heart (Fig. 3). It is most similar (93% identity) to a mosquito actin predicted from a genomic sequence (XM315269). It shared 87% to 90% identity with the cytoplasmic type and skeletal muscle type lobster actins. Three actins from the hepatopancreas, HA-ActinCT1, CT2, and CT3 were also more similar to each other than to other actins. HA-ActinCT2 and HA-ActinCT3 shared 98% amino acid sequence identity, while CT2 and CT3 shared ~95% identity with HA-ActinCT1.

The lobster actin sequences were compared with two actins from the Pacific white shrimp, *Litopenaeus vannamei* (Sun et al., 2007) and an actin from land crab, *Gecarcinus lateralis* (Varadaraj et al., 1996). Since it showed highest amino acid sequence identity (94%) to Ha-ActinSK3 and SK5, *L. vannamei* actin-T2 is likely a fast-type actin. *L. vannamei* actin-B is likely a cytoplasmic-type actin, as it shared 99% amino acid sequence identity with Ha-ActinCT1. The *G. lateralis* actin-1 is likely a slow-type actin, as it was isolated from the claw closer muscle, which contains S1 and S2 fibers (Mykles, 1988) and it was most similar to Ha-ActinSK1 and SK2 (Fig. 2).

A phylogenetic tree was constructed to reveal potential evolutionary relationships of the 12 lobster actins with actins from other species (Fig. 2). The 8 Ha-ActinSKs, Ha-ActinsHT1, *G. lateralis* actin-1, and *L. vannamei* actin-T2 were grouped into a muscle-type cluster with vertebrate muscle α-actins, whereas the 3 Ha-ActinsCTs, shrimp (*L. vannamei*-B), fruitfly (*D. melanogaster*-5C) actin, and 2 oyster (*C. gigas*-1 and -2) actins clustered with vertebrate β-actins. The grouping of Ha-ActinHT1 with Ha-ActinSK1-8 suggests that the cardiac and skeletal muscle actins evolved from an ancestral muscle-type gene. The Ha-ActinCTs appear to have evolved from a β-actin-like ancestor, with Ha-ActinCT2 and CT3 resulting from a later gene duplication event.

Actins have highly conserved sequences that mediate binding to myosin, tropomyosin, and other actin monomers (Hooper and Thuma, 2005; Kabsch and Vandekerckhove, 1992). The myosin head binds to the outer surface of subdomain 1 (Kabsch and Vandekerckhove, 1992; see Fig. 1 for locations of the subdomains). With reference to the unprocessed Ha-ActinSK1 sequence, the Glu95 is important for myosin binding (Razzaq et al., 1999). This residue is within an invariant six amino acid sequence (FYNELR), which is flanked by residues at positions #89, #91, and #98 that vary between isoforms (Fig. 1). Three other acidic residues (Glu363, Asp365, and Glu366) near the C-terminus have been shown to bind to myosin by chemical cross-linking (Sutoh, 1983). These are well conserved in the lobster actins, but are located within an eight-amino acid sequence that varies between isoforms (Fig. 1). The S201, T205, and Glu207 in one actin interact with Asp290 and Lys293 in the adjacent actin in the polymer

Table 3
Expression levels of twelve actin mRNAs in lobster skeletal muscles, heart, and hepatopancreas.

Isoform	Cr	Cu	DAF	DAE	Ht	Hp
SK1	35904 ± 4759*[a] (88%)	466 ± 231*[b] (1%)	8 ± 6	5 ± 2	0	0
SK2	4869 ± 837* (12%)	112 ± 62	0	0	0	0
SK3	2 ± 1	4099 ± 2647* (12%)	8 ± 4	2 ± 5	0	0
SK4	17 ± 14	16441 ± 6936* (48%)	37 ± 16	32 ± 15	1 ± 1	2 ± 1
SK5	2 ± 1	12726 ± 6102* (37%)	17149 ± 6642* (31%)	31118 ± 14748* (46%)	17 ± 7	2 ± 1
SK6	1 ± 0.1	407 ± 168* (1%)	422 ± 160* (1%)	782 ± 485 (1%)*	3 ± 1	1 ± 1
SK7	0	60 ± 59	1881 ± 1880 (3%)	160 ± 155	0	0
SK8	2 ± 1	205 ± 123 (1%)	35568 ± 6634* (65%)	35657 ± 21057* (53%)	93 ± 28 (1%)	12 ± 6
HT1	0	0	0	0	10886 ± 6621* (87%)	19 ± 6
CT1	27 ± 6	22 ± 6	29 ± 10	42 ± 25	1531 ± 638*a (12%)	622 ± 265*[b] (1%)
CT2	5 ± 0.2	4 ± 1	9 ± 5	4 ± 2	14 ± 3	47809 ± 24116* (93%)
CT3	4 ± 1	2 ± 2	2 ± 1	4 ± 3	15 ± 6	2758 ± 1731* (6%)
Total	40833 (100%)	34545 (100%)	55113 (100%)	67805 (100%)	12559 (100%)	51225 (100%)

Transcripts were quantified by real-time RT-PCR and normalized to 28S rRNA (see Materials and methods section). Data presented as mean relative copy number ± 1 S.D. (standard deviation) ($n = 3$). Percentages of each transcript in each tissue are given in parentheses, if at least 1% of the total. Asterisks indicate means significantly different ($P<0.05$) from means without asterisks for each actin isoform among different tissues; superscript letters (a, b) indicate means within the asterisked subset that are significantly different ($P<0.05$) from each other. For example, the means for SK1 in crusher (Cr) and cutter (Cu) claw muscle are significantly different from each other, and both means are significantly different from the means in deep abdominal extensor (DAE), deep abdominal flexor (DAF), hepatopancreas, (Hp), and heart (Ht).

(Kudryashov et al., 2005); all five residues are well conserved, except S201 is replaced T201 in the cytoplasmic type actins (Fig. 1). Three of four amino acid residues (positions #324–#327) involved in van der Waals contacts between actins vary between isoforms (Fig. 1). An α-helical sequence (Asp224 – Ser235) and three residues (Lys217, Lys240, and Pro309) are involved in tropomyosin binding (El-Saleh et al., 1984; Milligan and Flicker, 1987; Szilagyi and Lu, 1982). Lys217 is located within a highly conserved sequence, but Lys240 and Pro309 are located adjacent or within variable sequences (Fig. 1). Moreover, a five-amino acid sequence (positions #230–234) within the α-helix varies between the isoforms (Fig. 1). Residues involved in ATP binding are highly conserved (Fig. 1). These results suggest that the lobster actins retain key functional properties. However, variations in nearby residues may alter the protein binding properties of the different isoforms.

3.3. Tissue expression of twelve Ha-Actins

End-point RT-PCR was carried out to assess the qualitative expression pattern of the twelve actins in different tissues (Fig. 3). Both Ha-ActinSK1 and SK2 were expressed in the claw closer muscles. Ha-ActinSK3 and SK4 were expressed in the cutter claw closer muscle and at lower levels in the crusher claw closer muscle and deep abdominal flexor and extensor muscles. Ha-ActinSK5, SK6, SK7, and SK8 were expressed in cutter claw closer muscle and deep abdominal flexor and extensor muscles. Ha-ActinHT1 was expressed exclusively in heart. HA-ActinCT1 was expressed in all tissues, while Ha-ActinCT2 and CT3 were expressed in the hepatopancreas. These results support the classification of the lobster actins into nine muscle (8 skeletal muscle and 1 cardiac) and three cytoplasmic types.

The mRNA levels were quantified by real-time PCR (Table 3). Ha-ActinSK1 and SK2 appear to be "slow-type" actins, as they are expressed predominantly in the S1 fibers of the crusher claw. The other six skeletal muscle actins were expressed in fast muscles, although the relative levels differed between the cutter claw closer and the deep abdominal muscles. Ha-ActinSK3, SK4, and SK5 were the predominant isoforms in cutter claw, comprising 97% of the total actin mRNAs. In contrast, Ha-ActinSK5, SK6, SK7, and SK8 comprised 100% of the actin mRNAs in deep abdominal muscles. Moreover, the slow actins (SK1 and SK2) were expressed at low levels in cutter claw muscle, but not in deep abdominal muscles (Table 3). The muscles also differ in the mRNA levels of F and S1 myosin heavy chain isoforms and Ha-ActinSK1 (Medler and Mykles, 2003; Medler et al., 2005). These results suggest that fast fibers in cutter claw and abdomen constitute subtypes that differ in contractile properties. Ha-ActinHT1 was expressed exclusively in heart muscle, which supports its classification as a cardiac actin. All three cytoplasmic type actins were expressed at very low levels in skeletal muscle. Ha-ActinCT1 was expressed at its highest level in heart, while Ha-ActinCT2 and CT3 comprised 99% of the actins in hepatopancreas. This indicates that Ha-ActinCT2 is the major isoform in non-muscle tissues.

This study has revealed an unprecedented diversity of actin isoforms expressed in crustacean tissues. The functional significance of this diversity remains to be established. It is the assemblage of myofibrillar protein isoforms that determines the contractile properties, such as Ca^{2+} sensitivity, tension, and contraction speed, of muscle fibers. Since only one muscle actin was previously identified (Koenders et al., 2002), it was assumed that the isoforms of myosin, tropomyosin, and troponin were responsible for the differences between F, S1, and S2 fibers in lobster (Cotton and Mykles, 1993; Medler and Mykles, 2003; Medler et al., 2004, 2005; Mykles 1985a,b, 1988; Mykles et al., 1998). However, the fiber type-specific expression of the eight skeletal muscle actins suggests that actin isoforms also contribute to contractile function. It is first necessary to determine how the expression of the twelve actins is correlated with the expression of the isoforms of myosin heavy chain, tropomyosin, and troponin. Second, it is necessary to determine which actins are involved in the remodeling of fibers during molt-induced atrophy and fiber transformation. Molting hormone (ecdsteroids) has little effect on Ha-ActinSK1 expression in lobster claw closer and deep abdominal muscles (Medler et al., 2005), but expression of at least some of the other isoforms may be affected. In summary, we isolated twelve cDNAs encoding actins that displayed different structural characteristics and tissue expression patterns. These results and previous studies suggest that fast fibers in the cutter claw closer and deep abdominal muscles constitute discrete subtypes.

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