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Cloning and mRNA Expression of an Actin cDNA from the Mulberry Longicorn Beetle, *Apriona germari*

Zhongzheng Gui, Kwang Sik Lee, Yadong Wei, Hyung Joo Yoon[1], Iksoo Kim[1], Xijie Guo[2], Hung Dae Sohn and Byung Rae Jin*

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.
[1]Department of Agricultural Biology, National Institute of Agricultural Science & Technology, Suwon 441-100, Korea.
[2]Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, P. R. China.

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Actin is a ubiquitous and highly conserved protein found in eukaryotic organisms. In this study, we describe the cDNA cloning and mRNA expression of an actin gene from the mulberry longicorn beetle, *Apriona germari*. The *A. germari* actin cDNA is 1524 bp containing a complete 1128 bp open reading frame that encodes a polypeptide of 376 amino acid residues with a predicted molecular weight of about 41.5 kDa. The deduced amino acid sequence of the *A. germari* actin cDNA showed 99% protein sequence identity to *Homalodisca coagulata* actin, differing at only two amino acid positions, and 92 – 98% protein sequence identity to known insect species actins. The predicted three-dimensional structure of *A. germari* actin revealed the four residue hydrophobic pulg loop characteristic of the actin family. Northern blot analysis showed that *A. germari* actin is highly expressed in epidermis and muscle, and less strongly in midgut, but not in the fat body of *A. germari* larva.

Key words: *Apriona germari*, cDNA cloning, Actin, mRNA expression, Mulberry longicorn beetle

Introduction

Actin is a major contractile protein found in all eukaryotic cells. As the major component of thin filaments, actin is one of the primary proteins responsible for muscle contraction. Furthermore, actin is involved in many cellular processes such as cell motility, cytokinesis and morphogenesis (Pollard and Cooper, 1986).

Actin is a ubiquitous and highly conserved protein, which in many organisms is encoded by multigene families in which individual isoforms probably perform cell-specific functions (Rubenstein, 1990). In insect, the actin gene structure and its expression have been studied extensively in *Drosophila melanogaster* (Bernstein *et al.*, 1993; Mounier and Sparrow, 1993; Lovato *et al.*, 2001). The *D. melanogaster* actin gene family consists of six highly conserved genes that exhibit both developmental stage- and tissue-specific expression (Fyrberg *et al.*, 1980; Tobin *et al.*, 1980). The expression patterns of the individual genes have suggested differing functional roles for the various actins. In *D. melanogaster*, two actin genes (*Act5C* and *Act42A*) are cytoplasmic, based upon its ubiquitous expression during embryogenesis (Burn *et al.*, 1989; Tobin *et al.*, 1990) and the other four actin genes (*Act57B, Act87E, Act79B* and *Act88F*) are predominantly muscle isoforms, but each is unique in its temporal and spatial expression patterns (Fyrberg *et al.*, 1983; Hiromi and Hotta, 1985; Ball *et al.*, 1987; Courchesne-Smith and Tobin, 1989; Tobin *et al.*, 1990; Keller *et al.*, 1997).

In insects, actin genes have been isolated from only three order species such as Diptera, Lepidoptera, and Hemiptera. The purpose of the present study was to elucidate the actin gene in the mulberry longicorn beetle, *Apriona germari* (Coleoptera: Cerambycidae). In this paper, we reported the cDNA cloning and mRNA expression of the *A. germari* actin gene in coleopteran insect for the first time.

Materials and Methods

Insects

The larvae of the mulberry longicorn beetle, *Apriona ger-*

*To whom correspondence should be addressed.
College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea. Tel: +82-51-200-7594;
Fax: +82-51-200-7594; E-mail: brjin@daunet.donga.ac.kr

mari (Coleoptera: Cerambycidae), were reared on an artificial diet as described previously (Yoon and Mah, 1999).

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Kim *et al.*, 2001) constructed using whole bodies of *A. germari* larvae was used in this study. The clones harboring cDNA inserts were randomly selected and sequenced to generate the expressed sequence tags (ESTs) (Kim *et al.*, 2003). The plasmid DNA was extracted by Wizard mini-preparation kit (Promega, Madison, WI). The nucleotide sequence was determined by using a BigDyeTerminator cycle sequencing kit and an automated DNA sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI (http://www.ncbi.nlm.nih.gov/BLAST). GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program. MacVector (ver. 6.5, Oxford Molecular Ltd) was used to align the amino acid sequences of actin. To generate structural models, amino acid sequences of *A. germari* actin cDNA were submitted to Swiss-Model (Schwede *et al.*, 2003) using the First Approach Method set at default parameters. Homology models were generated using the known structure of the *Bos taurus* profilin-beta-actin (Protein Data Bank code No. 2btf; Schutt *et al.*, 1993), *B. taurus* beta-actin (Protein Data Bank code No. 1hlu; Chik *et al.*, 1996), and *Oryctolagus cuniculus* actin (Protein Data Bank code No.1ijj; Bubb *et al.*, 2002). Swiss-Pdb viewer version 3.7 was used to generate a three-dimensional image.

RNA isolation and Northern blot analysis

The *A. germari* larva was dissected under the Stereomicroscope (Zeiss, Jena, Germany), individual samples such as midgut, fat body, epidermis, and muscle 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 the midgut, fat body, epidermis, and muscle of *A. germari* larva by using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from *A. germari* was denatured by glyoxalation (McMaster and Carmicharl, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) 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 1524 bp actin 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 cloning, sequencing, phylogenetic analysis, and molecular modeling of *A. germari* actin

In a search of *A. germari* ESTs (expressed sequence tags), we identified a cDNA showing high homology with previously reported actin genes. The cDNA clone contains its complete coding region as well as 5' and 3' untranslated

```
-45                GGTGTTTTTCTGTCTAGTGAGCAGTGAAAAACCACATCAGACAAC
  1  ATGTGTGACGACGATGTTGCGGCTCTTGTCGTTGACAATGGTTCCGGAATGTGCAAAGCC
     M  C  D  D  D  V  A  A  L  V  V  D  N  G  S  G  M  C  K  A
 61  GGTTTCGCCGGTGATGATGCACCCCGCGCCGTCTTTCCATCCATCGTCGGTCGCCCCAGG
     G  F  A  G  D  D  A  P  R  A  V  F  P  S  I  V  G  R  P  R
121  CATCAGGGAGTAATGGTCGGTATGGGACAAAAAGACTCTTACGTAGGAGATGAAGCCCAA
     H  Q  G  V  M  V  G  M  G  Q  K  D  S  Y  V  G  D  E  A  Q
181  AGCAAAAGAGGTATCCTCACTTTGAAATACCCCATTGAACACGGCATCATTACCAACTGG
     S  K  R  G  I  L  T  L  K  Y  P  I  E  H  G  I  I  T  N  W
241  GACGATATGGAAAAGATCTGGCATCACACCTTCTACAATGAACTCCGTGTTGCCCCAGAA
     D  D  M  E  K  I  W  H  H  T  F  Y  N  E  L  R  V  A  P  E
301  GAACACCCAGTCCTTCTTACTGAAGCTCCCCTCAACCCCAAGGCTAACCGTGAAAAGATG
     E  H  P  V  L  L  T  E  A  P  L  N  P  K  A  N  R  E  K  M
361  ACTCAAATCATGTTTGAAACCTTCAACACACCCGCTATGTATGTTGCCATCCAAGCTGTA
     T  Q  I  M  F  E  T  F  N  T  P  A  M  Y  V  A  I  Q  A  V
421  CTTTCCCTATACGCTTCTGGTCGTACCACTGGTATTGTATTGGACTCTGGAGATGGTGTC
     L  S  L  Y  A  S  G  R  T  T  G  I  V  L  D  S  G  D  G  V
481  ACTCACACTGTACCAATTTATGAAGGTTACGCTCTTCCCCATGCCATCCTCCGTTTGGAC
     T  H  T  V  P  I  Y  E  G  Y  A  L  P  H  A  I  L  R  L  D
541  TTGGCTGGCCGTGACTTGACCGACTACCTCATGAAAATCCTTACCGAAAGAGGCTACTCA
     L  A  G  R  D  L  T  D  Y  L  M  K  I  L  T  E  R  G  Y  S
601  TTCACCACCACGGCTGAAAGGGAAATTGTTCGTGACATCAAGGAAAAACTTTGCTATGTT
     F  T  T  T  A  E  R  E  I  V  R  D  I  K  E  K  L  C  Y  V
661  GCCCTCGACTTTGAACAGGAAATGGCCACCGCCGCCGCCTCAACCTCCCTCGAAAAGAGC
     A  L  D  F  E  Q  E  M  A  T  A  A  A  S  T  S  L  E  K  S
721  TACGAACTTCCTGATGGACAGGTCATCACCATCGGTAACGAAAGATTCCGTTGCCCTGAA
     Y  E  L  P  D  G  Q  V  I  T  I  G  N  E  R  F  R  C  P  E
881  GCTCTATTCCAGCCTTCCTTCTTGGGTATGGAATCTTGCGGTATCCATGAAACCGTATAC
     A  L  F  Q  P  S  F  L  G  M  E  S  C  G  I  H  E  T  V  Y
941  AACTCCATCATGAAGTGCGACGTTGATATCCGTAAGGACTTGTACGCCAACACTGTACTC
     N  S  I  M  K  C  D  V  D  I  R  K  D  L  Y  A  N  T  V  L
1001 TCCGGAGGCACCACCATGTACCCTGGTATTGCTGACCGTATGCAAAAGGAAATCACAGCC
     S  G  G  T  T  M  Y  P  G  I  A  D  R  M  Q  K  E  I  T  A
1061 CTTGCCCCATCCACCATCAAGATCAAGATCATTGCTCCCCCAGAAAGGAAATACTCCGTA
     L  A  P  S  T  I  K  I  K  I  I  A  P  P  E  R  K  Y  S  V
1121 TGGATCGGGGGATCCATCTTGGCTTCCCTCTCTACCTTCCAACAGATGTGGATCTCCAAG
     W  I  G  G  S  I  L  A  S  L  S  T  F  Q  Q  M  W  I  S  K
1181 CAAGAATACGACGAATCCGGCCCTGGAATTGTCCACCGCAAGTGCTTCTAAGCGATTTAA
     Q  E  Y  D  E  S  G  P  G  I  V  H  R  K  C  F  *
1241 TTGTATTTCTAATAAGCTATCCGACATGTTGTTATACTAGTATTACATCTACAGTTTTAT
1301 TATTAATGCGACCGTGGTTGTACTGCCAGACAGACTTTTATTTGAACATCATATCAATTA
1361 ACACCTTTTGATTATTATGTTTATTATTTGTTATTATTATACTTTACTTATTGTAATTAT
1421 TTTACTCACTGGGCAGGTCAATAAAGCCTATCTAGTAATAAAAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of the *A. germari* actin cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. In the cDNA sequence, the polyadenylation sequence AATAAA is underlined. This cDNA sequence has been deposited in GenBank under accession number AY817141.

sequences. The nucleotide and its deduced amino acid sequences of the cDNA encoding actin are presented in Fig. 1. The entire length of *A. germari* actin cDNA is 1524 bp containing a complete 1128 bp open reading frame (ORF) that encodes a polypeptide of 376 amino acid residues with a predicted molecular weight of about 41.5 kDa.

The alignment of the deduced protein sequence of *A. germari* actin gene with available insect actin sequences is

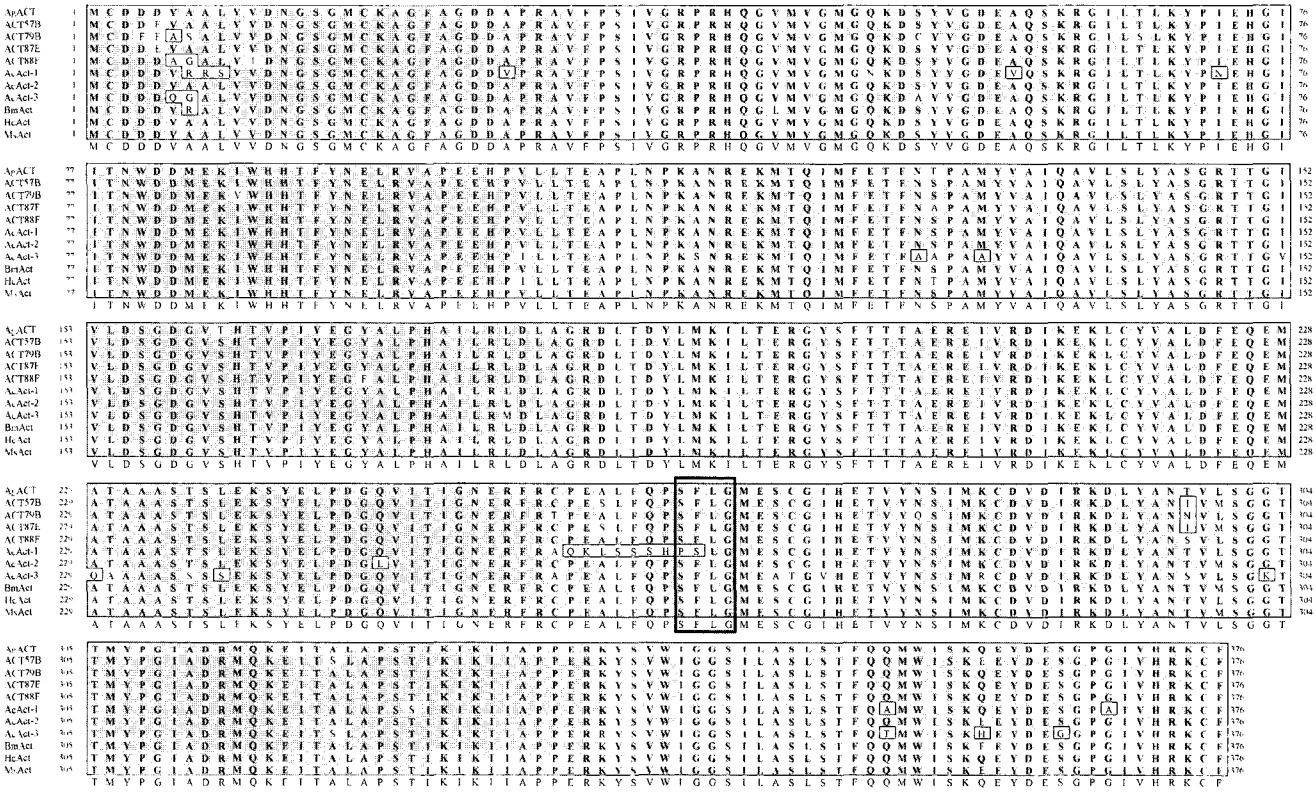

Fig. 2. Alignment of the amino acid sequence of *A. germari* actin with known insect actins. Residues are numbered according to the aligned insect actin sequences, and invariant residues are shaded black. The four residue (266-269) hydrophobic plug loop is boxed. The abbreviation and GenBank accession number for the actin sequences aligned are: AgACT, *A. germari* actin (AY817141; this study); Act57B (NM079076), Act79B (NM079486), Act87E (NM169525) and Act88F (NM079643), *Drosophila melanogaster* actin isoforms; AeAct-1 (U20287), AeAct-2 (AY289764) and AeAct-3 (AY289765), *Aedes aegypti* actin isoforms; BmAct, *Bombyx mori* actin (P07836); HcAct, *Homalodisca coagulata* actin (AY588061); MsAct, *Manduca sexta* actin (P49871).

Percent similarity

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11
1. AgAct	This Study		98	98	99	99	95	99	96	99	99	99
2. Act57B	NM_079076	97		98	99	98	94	98	96	99	98	98
3. Act79B	NM_079486	96	96		98	99	94	98	96	98	98	98
4. Act87E	NM_169525	98	99	96		98	94	99	96	99	99	99
5. Act88F	NM_079643	98	97	97	97		94	98	97	98	99	99
6. AeAct-1	U20287	94	93	92	93	93		94	93	95	95	95
7. AeAct-2	AY289764	98	98	96	98	97	93		96	99	99	99
8. AeAct-3	AY289765	92	92	91	92	93	88	92		96	97	96
9. BmAct	P07836	98	98	96	98	97	94	99	92		99	99
10. HcAct	AY588061	99	97	96	98	98	94	98	93	98		99
11. MsAct	P49871	98	98	96	98	98	94	99	92	99	98	

Percent identity

Fig. 3. Pairwise identities and similarities of the deduced amino acid sequence of *A. germari* actin among insect actin sequences. The abbreviation and GenBank accession number for the actin sequences aligned are described in Fig. 2 legend.

shown in Fig. 2. The alignment result indicates that *A. germari* actin sequence is closely related to known actins derived from insects. The *A. germari* actin size encoding 376 amino acid residues is identical in all five species, suggesting that the protein-coding region and its size are highly conserved in the actins from insects.

Fig. 3 shows the similarity and identity of the deduced protein sequence of *A. germari* actin cDNA with those of insect actin sequences. The *A. germari* actin sequence was most identical to *Homalodisca coagulata* actin (99% protein sequence identity), differing at only two amino acid positions, 104 and 161. In addition, the deduced amino acid sequence of *A. germari* actin cDNA showed 92 – 98% protein sequence identity to known insect species actins. These similarities prompted us to that *A. germari* actin cDNA in this study encodes a putative actin family. Actin is a ubiquitous and highly conserved eukaryotic protein, with only a few amino acid sequence differences between species as evolutionarily distant as humans and slime molds (Fyrberg *et al.*, 1981; Rubenstein, 1990; Lovato *et al.*, 2001; Wagner *et al.*, 2002). It is critical for cell movement, determination of cell shape and cell division, and it plays important roles in many other cellular processes, including organelle transport. As described previously in insect actins, actins have several isoforms, which are coexpressed in most cell types and have very similar sequences to each other (Macias and Sastre, 1990; Lovato *et al.*, 2001; Krebs *et al.*, 2002; Wagner *et al.*, 2002).

The *A. germari* actin model shows that the monomer contains the hydrophobic plug loop at amino acid positions, 266 – 269 (Fig. 4). This loop includes the four residues of hydrophobic plug that is believed to insert into a hydrophobic pocket formed by two adjacent monomers on the opposing strand, thereby stabilizing the F-actin helix (Holmes *et al.*, 1990; Lovato *et al.*, 2001). The four amino acid residues of hydrophobic plug were well conserved in the insect actins except for *A. aegypti* actin isoform AeAct-1.

mRNA expression of *A. germari* actin

To confirm the expression of *A. germari* actin gene at transcriptional level, Northern blot analysis was performed using mRNA prepared from midgut, fat body, epidermis and muscle, respectively (Fig. 5). Hybridization signal was detected as a single band in mRNA from epidermis, muscle and midgut. The signal of *A. germari* actin transcripts showed the strongest band in the epidermis and next in the muscle, while a weak band was found in midgut. The result showed that the *A. germari* actin cloned in this study was predominantly detected in the epidermis, suggesting that the epidermis is a predominant expression site for the actin.

In *Drosophila*, transcripts of the six actin genes accumulate in a stage- and tissue-specific manner (Fyrberg *et al.*, 1983) and the actin isoforms showed varied levels of expression during development, consistent with roles in muscle development (Courchesne-Smith and Tobin, 1989; Lovato *et al.*, 2001). The expression profile of isoform actins was detected in tubular muscles of the ventral thorax, in the internal muscles of the leg, in the abdomen in the body wall muscles, and in the visceral muscles surrounding the gut. In addition, two isoforms each of cyto-

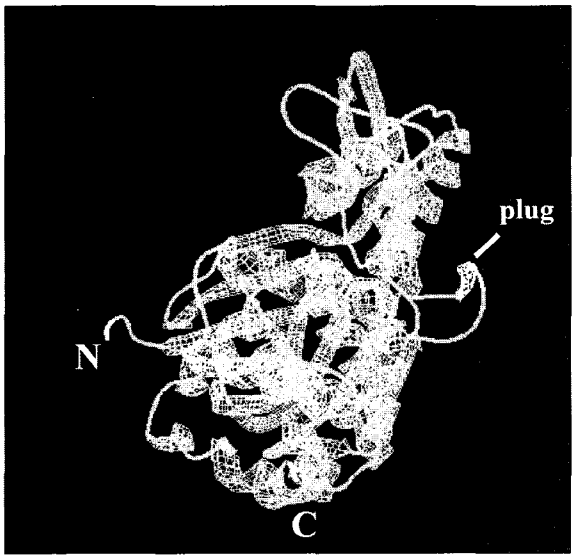

Fig. 4. Predicted three-dimensional structure of *A. germari* actin. N, N-terminus; C, C-terminus; plug, four residue (266-269) hydrophobic plug loop.

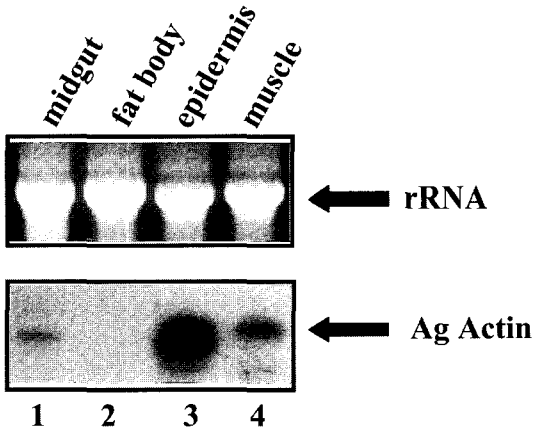

Fig. 5. Northern blot analysis of *A. germari* actin gene. Total RNA was isolated from the midgut (lane 1), fat body (lane 2), epidermis (lane 3), and muscle (lane 4) of *A. germari* larva, respectively. The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred on to a nylon membrane, and hybridized with radiolabelled 1524 bp *A. germari* actin cDNA (lower panel).

plasmic actin in *D. melanogaster*, larval muscle actin and adult muscle actin have been identified, all encoded by different genes (Fyrberg *et al.*, 1980, 1981).

The present result represents the first complete actin cDNA from the mulbeery longicorn beetle, *A. germari*. Further studies would be required in order to characterize the actin cytoskeletal function in the beetle with many different cell types and actin structures with a genetic approach.

Acknowledgements

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References

Ball, E., C. C. Karlik, C. J. Beall, D. L. Saville, J. C. Sparrow, B. Bullard and E. A. Fyrberg (1987) Arthrin, a myofibrillar component of insect flight muscle, is an actin-ubiquitin conjugate. *Cell* **51**, 221-228.

Bernstein, S. I., P. T. O'Donell and R. M. Cripps (1993) Molecular genetic analysis of muscle development, structure and function in *Drosophila*. *Int. Rev. Cytol.* **43**, 63-152.

Bubb, M. R., L. Govindasamy, E. G. Yarmola, S. M. Vorobiev, S. C. Almo, T. Somasundaram, M. S. Chapman, M. Agbandje-McKenna and R. McKenna (2002) Polylysine induces an antiparallel actin dimmer that nucleates filament assembly: crystal structure at 3.5-A resolution. *J. Biol. Chem.* **277**, 20999-21006.

Burn, T. C., J. O. Vigoreaux and S. L. Tobin (1989) Alternative 5C actin transcripts are localized in different patterns during *Drosophila* embryogenesis. *Dev. Biol.* **131**, 345-355.

Chik, J. K., U. Lindberg and C. E. Schutt (1996) The structure of an open state of beta-actin at 2.65A resolution. *J. Mol. Biol.* **263**, 607-623.

Courchesne-Smith, C. L. and S. L. Tobin (1989) Tissue-specific expression of the 79B actin gene during *Drosophila* deveopment. *Dev. Biol.* **133**, 313-321.

Fyrberg, E. A., K. L. Kindle, N. Davidson and A. Sodja (1980) The actin genes of *Drosophila*: a dispersed multigene family. *Cell* **19**, 365-378.

Fyrberg, E. A., B. J. Bond, N. D. Hershey, K. S. Mixter and N. Davidson (1981) The actin genes of *Drosophila*: protein coding regions are highly conserved but intron positions are not. *Cell* **24**, 107-116.

Fyrberg, E. A., J. W. Mahaffey, B. J. Bond and N. Davidson (1983) Transcripts of the six *Drosophila* actin genes accumulate in a stage- and tissue-specific manner. *Cell* **33**, 115-123.

Hiromi, Y. and Y. Hotta (1985) Actin gene mutations in *Drosophila*: heat shock activation in the indirect flight muscles. *EMBO J.* **4**, 1681-1687.

Holmes, K. C., D. Popp, W. Gebhard and W. Kabsch (1990) Atomic model of the F-actin structure. *Nature* **347**, 44-49.

Keller, C. A., M. S. Erickson and S. M. Abmayr (1997) Misexpression of *nautilus* induces myogenesis in cardioblasts and alters the pattern of somatic muscle fibers. *Dev. Biol.* **181**, 197-212.

Kim, S. R., H. J. Yoon, N. S. Park, S. M. Lee, J. Y. Moon, B. R. Jin and H. D. Sohn (2001) Molecular cloning of a cDNA encoding a cathepsin D homologue from the mulberry longicorn beetle, *Apriona germari*. *Int. J. Indust. Entomol.* **3**, 121-126.

Kim, S. R., H. J. Yoon, K. S. Lee, I. Kim, Y. H. Je, H. D. Sohn and B. R. Jin (2003) Molecular cloning of three cDNAs encoding putative larval cuticle protein expressed differentially after larval ecdysis from the mulberry longicorn beetle, *Apriona germari*. *Comp. Biochem. Physiol. B* **136**, 803-811.

Krebs, K. C., K. L. Brzoza and Q. Lan (2002) Use of subtracted libraries and macroarray to isolate developmentally specific genes from the mosquito, *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **32**, 1757-1767.

Lovato, T. L., S. M. Meadows, P. W. Baker, J. C. Sparrow and R. M. Cripps (2001) Characterization of muscle actin genes in *Drosophila virilis* reveals significant molecular complexity in skeletal muscle types. *Insect Mol. Biol.* **10**, 333-340.

Macias, M. T. and L. Sastre (1990) Molecular cloning and expression of four actin isoforms during *Artemia* development. *Nucl. Acids Res.* **18**, 5219-5225.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Pollard, T. D. and J. A. Cooper (1986) Actin and actin-binding protein. A critical evaluation of mechanisms and functions. *Ann. Rev. Biochem.* **55**, 987-1035.

Rubenstein, P. A. (1990) The functional importance of multiple actin isoforms. *Bioessays* **12**, 309-315.

Schutt, C. E., J. C. Myslik, M. D. Rozycki, N. C. Goonesekere and U. Lindberg (1993) The structure of crystalline profilin-beta-actin. *Nature* **365**, 810-816.

Schwede, T., J. Kopp, N. Guex and M. C. Peitsch (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucl. Acids Res.* **31**, 3381-3385.

Tobin, S. L., P. J. Cook and T. C. Burn (1990) Transcripts of individual *Drosophila* actin genes are differentially distributed during embryogenesis. *Dev. Genet.* **11**, 15-26.

Tobin, S. L., E. Zulauf, F. Sanchez, E. A. Craig and B. J. McCarthy (1980) Multiple actin-related sequences in the *Drosophila melanogaster* genome. *Cell* **19**, 121-131.

Wangner, C. R., A. P. Mahowald and K. G. Miller (2002) One of the two cytoplasmic actin isoforms in *Drosophila* is essential. *Proc. Natl. Acd. Sci. USA* **99**, 8037-8042.

Yoon, H. J. and Y. I. Mah (1999) Life cycle of the mulberry longicorn beetle, *Apriona germari* Hope on an artificial diet. *J. Asia-Pacific Entomol.* **2**, 169-173.