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Molecular Cloning of a Profilin cDNA from *Bombyx mori*

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The actin-binding protein profilin cDNA was firstly isolated from the lepidopteran insect, silkworm *Bombyx mori*. The *B. mori* profilin cDNA contains an open reading frame of 378 bp encoding 126 amino acid residues and possesses three cysteine residues. The deduced amino acid sequence of the *B. mori* profilin cDNA showed 80% identity to *Apis mellifera* profilin and 72% to *Drosophila melanogaster* profilin. Northern blot analysis showed that *B. mori* profilin is highly expressed in epidermis and less strongly in silk gland. In addition, Northern blot analysis revealed the presence of *B. mori* profilin transcripts in all tissues examined, suggesting that *B. mori* profilin gene is expressed in most, if not all, body tissues.

Key words: Actin-binding protein, *Bombyx mori*, cDNA, Insect, Profilin, Silkworm

Introduction

Profilin is a actin monomer-binding protein with a molecular mass of 12 – 15 kDa that was originally identified in calf spleen (Carsson *et al.*, 1977) and subsequently found in animal cells (Reichstein and Korn, 1979), plants (Valenta *et al.*, 1991), and viruses (Machesky *et al.*, 1994). The profilin is thought to be a key regulator of actin polymerization in cells (Theriot and Mitchison, 1993). Actin and its associated proteins are fundamental elements of the cytoskeleton which together play an important role in morphogenesis, mitogenesis, movement and other cellular processes in eukaryotic cells (Staiger and Schliwa, 1987; Aderem, 1992; Lauffenburger and Hortwitz, 1996; Mitchson and Cramer, 1996). Actin polymerization is important for a wide range of cellular functions and properties, including cell division, cell motility, cell polarity and cell-cell contacts, and profilins are involved in the regulation of actin dynamics (Sun *et al.*, 1995; Suetsugu *et al.*, 1998).

Profilins are ubiquitous proteins found in eukaryotic cells. The important of profilins in various organisms has been shown by knockout studies in *Dictyostelium discoideum* (Haugwitz *et al.*, 1991), *Drosophila* (Verheyen and Colley, 1994), yeast (Haarer *et al.*, 1990; Balasubramanian *et al.*, 1994) and mice (Witke *et al.*, 2001), where severe phenotypes related to compromised actin polymerization was demonstrated. Prolifin exists as two isoforms in mammals, prolifins I and II. Prolifin I is more ubiquitous and abundant than prolifin II (Honore *et al.*, 1993; Witke *et al.*, 1998) and is essential for cell survival and cell division in early mouse development (Witke *et al.*, 2001). In insect, a recent study reported that a balance of capping protein and profiling functions is required to regulate actin polymerization in *Drosophila* bristle (Hopmann and Miller, 2003). In the gene level insects, profilin genes have been isolated from only *D. melanogaster* and *A. mellifera* (Cooley *et al.*, 1992; Kucharski and Maleszka, 2004). The purpose of the present study was to elucidate the prolifin gene in the silkworm, *B. mori* as a model insect for lepidopteran insect. In this paper, we reported the cDNA cloning and mRNA expression of a silkworm profilin for the first time.

Materials and Methods

Animals

The larvae of the silkworm, *Bombyx mori*, used in this study were Chinese race Jam 108 supplied by Department of Sericulture and Entomology, The National Institute of Agricultural Science and Technology, Korea. Silkworms

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were reared on fresh mulberry leaves at 25°C, 65 ± 5% of relative humidity, and 12 hrs light : 12 hrs dark photoperiod as usual.

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Kim *et al.*, 2003) constructed using whole bodies of *B. mori* 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 in the 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 profilin. With the two GenBank-registered insect profilin amino acid sequences, phylogenetic analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 (Swofford, 2000). The accession numbers of the sequences in the GenBank are as follows: *Bombyx mori* (AY690617; this study), *D. melanogaster* profilin (AAA28418), and *A. mellifera* profilin (AAS50519).

RNA isolation and Northern blot analysis

The larvae of *B. mori* were dissected under the Stereomicroscope (Zeiss, Jena, Germany), individual samples such as fat body, midgut, silk gland, and epidermis were harvested, and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNA was isolated from the fat body, midgut, silk gland, and epidermis of *B. mori* larvae by using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from *B. mori* 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 1,463 bp profilin cDNA clone was labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene) 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 and phylogenetic analysis of *B. mori* profilin

In a search of B. mori ESTs (expressed sequence tags), we identified a cDNA showing high homology with previously reported profilin genes. The cDNA clone including the full-length open reading frame (ORF) was sequenced and characterized. The nucleotide and its deduced amino acid sequences of the cDNA encoding profilin are presented in Fig. 1. The entire length of B. mori profilin cDNA is 1,463 bp containing a complete 378 bp ORF that encodes a polypeptide of 126 amino acid residues with a predicted molecular weight of about 14 kDa.

The alignment of the deduced protein sequence of *B. mori* profilin gene with available insect profilin sequences indicates that *B. mori* profilin sequence is closely related to *A. mellifera* profilin (Kucharski and Maleszka, 2004) and *D. melanogaster* profilin (Cooley *et al.*, 1992) (Figs. 2 and 3). The *B. mori* profilin also has three conserved cys-

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-78                                                    gcacgagggagcgtttgc
    tcgttgtgaggagtatcttgtgtttaagtgtagttttatttttaattgaacttaatcaaa
  1 ATGAGCTGGCAAGATTATGTCGACAAACAGTTAATGGCATCTAGATGTGTCACAAAAGCT
  1 M  S  W  Q  D  Y  V  D  K  Q  L  M  A  S  R  C  V  T  K  A
                                                   ▲
 61 GCCATTGCCGGTCATGATGGCAATGTGTGGGCAAAGTCGGAAGGCTTCGAAATTTCAAAA
 21 A  I  A  G  H  D  G  N  V  W  A  K  S  E  G  F  E  I  S  K
121 GATGAAGTGGCGAAGATTGTGGCTGGCTTTGAGAATGAATCACTGCTAACGAGTGGCGGC
 41 D  E  V  A  K  I  V  A  G  F  E  N  E  S  L  L  T  S  G  G
181 GTGACGATAGCGGGCACGCGGTACATCTACCTCAGTGGCACAGACCATATCATCCGCGCG
 61 V  T  I  A  G  T  R  Y  I  Y  L  S  G  T  D  H  I  I  R  A
241 AAGCTTGGCAAGGTCGGCGTGCATTGCATGAAGACACAGCAAGCTGTGGTCATTTCTCTC
 81 K  L  G  K  V  G  V  H  C  M  K  T  Q  Q  A  V  V  I  S  L
                              ▲
301 TATGAAGAACCCATCCAACCTCAGCAGGCCGCATCTGTCGTGGAGAAGTTAGGAGAATAT
101 Y  E  E  P  I  Q  P  Q  Q  A  A  S  V  V  E  K  L  G  E  Y
361 TTAATTACCTGTGGTTATTAGgaggtcgaagctcgcccgtgatctaagagtactataaga
121 L  I  T  C  G  Y*
                 ▲
421 taatatttttccacggggaaataagtaactgcattttgtatattctataaagtgataag
481 aggaccgttcatttgttttgatattcgataaattatggcggcgtaatcagtgtgatgagt
541 ttagtgtgcacatgtgattattttgtggattctggacccttttttatacgctcaccgtctc
601 ctcaaaataattattactgcataatgaattaaatttgtattaaaatttgcttttattga
661 ccagtcatagtgaagcatttttagctttgcatagctgatctcaaacaaagcatgaagtat
721 agagatttttaggcttcttatcaagcatcattgtacgtaagtaacttcgcggactgctaa
781 gctgttgttcagttggacatagcacaaaaaccggtatagcttcatgattgtttcttgtac
841 tggatgcttgataagaatcttttaatatctcccgtaatgaaattacgcgtgacaacattt
901 tacaacattatatatatatatatatttataaaataagcatacatctaagggcttgttacaa
961 atttaaaggcactgaaacccaagtcttacaatttttaattaaaaatctctagtcccactgg
1021 aaccatcgtgtgaatttacatccaagcacaagcggcggcagcgcaggactcgcgccgtaa
1081 taacctttgctatttcaaaaatctgctatgctttgtttttatttttgccgcattttata
1141 atcatattatgtagtagaacataaacattttgcattctgtttaaaactgtcttcttagct
1201 gcagacaatagaagcgctcaattaattaccctataacctcagaaaaggcttgcagaaaat
1261 cgaaggggtgcatattgtttatatttgatattactactatagctttgtacattgtttctg
1321 ataagccgatgggaactgtgtctatctgataataaatgatttgtattataaaaaaaaaa
1381 aaaaa
```

Fig. 1. The nucleotide and deduced protein sequences of the *B. mori* profilin cDNA. The start codon of ATG is boxed and the termination codon is shown by asterisk. In the cDNA sequence, the polyadenylation sequence and poly(A) tail are shad-boxed and underlined, respectively. The conserved cysteine residues are shown by triangles. This cDNA sequence has been deposited in GenBank under accession number AY690617.

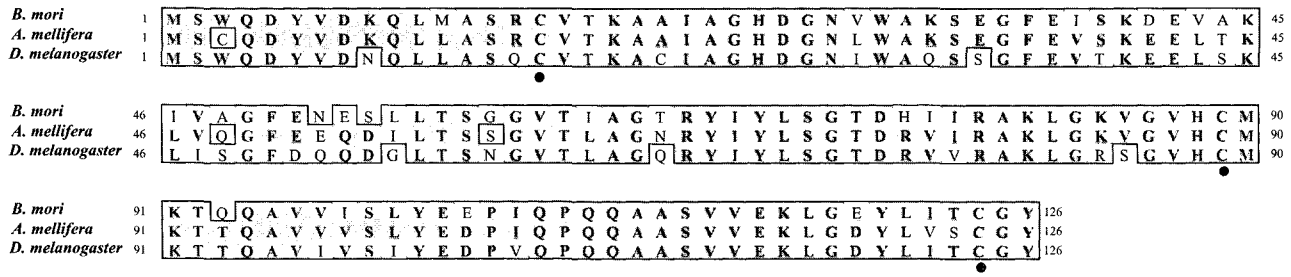
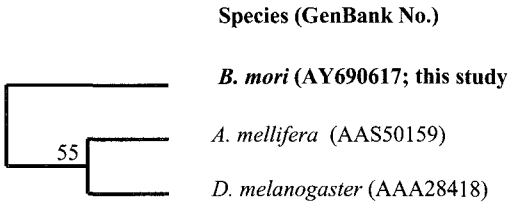


Fig. 2. Alignment of the amino acid sequence of *B. mori* profilin with known insect profilins. Residues are numbered according to the aligned insect profilin sequences, and invariant residues are shaded black. The conserved cysteine residues are shown by circles. The insect profilin sequences were taken from the following sources: *Drosophila melanogaster* profilin (AAA28418), and *Apis mellifera* profilin (AAS50519).

(A)



(B)

species	GenBank No.	Percent similarity 1	2	3
1. *B. mori*	this study		94	94
2. *A. mellifera*	AAS50159	80		93
3. *D. melanogaster*	AAA28418	72	80	

Percent identity

Fig. 3. Phylogenetic relationships among insect profilin sequences. (A) The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. GenBank accession numbers of the sequences used in the comparison are: *Bombyx mori* (this study), *Drosophila melanogaster* profilin (AAA28418), and *Apis mellifera* profilin (AAS50519). (B) Pairwise identities and similarities of the deduced amino acid sequence of *B. mori* profilin among insect profilin sequences.

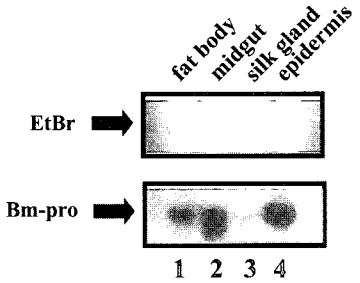


Fig. 4. Northern blot analysis of *B. mori* profilin. Total RNA was isolated from the fat body (lane 1), midgut (lane 2), silk gland (lane 3), and epidermis (lane 4) of *B. mori* larva, respectively. The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred on to a nylon membrane, and hybridized with radiolabelled 1,463 bp *B. mori* profilin cDNA (lower panel).

teines. The profilin gene size is identical in three insect species and encodes 126 amino acid residues, which is conserved in the insect profilins. The deduced protein sequence of the *B. mori* profilin showed 80% and 72% protein sequence identity to *A. mellifera* profilin and *D. melanogaster* profilin, respectively (Fig. 3B).

mRNA expression of *B. mori* profilin

To confirm the expression of *B. mori* profilin gene at transcriptional level, Northern blot analysis was performed using mRNA prepared from fat body, midgut, silk gland and epidermis, respectively (Fig. 4). Hybridization signal was present in all tissues examined, suggesting that *B. mori* profilin gene is expressed in most, if not all, body tissues. The signal of *B. mori* profilin transcripts in epidermis showed a strong band, while a weak band was found in silk gland. In human, the profilin II transcripts are expressed in all the tissues and cell lines and the levels of expression are generally complementary to the levels of the profilin I transcripts (Honore *et al.*, 1993). Further biochemical studies are necessary to reveal the exact physiological role of *B. mori* profilin.

Acknowledgements

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