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Research Paper

Cloning and sequence analysis of β-actin gene from Aedes albopictus (Diptera: Culicidae)☆

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Abstract

Objective: To obtain the complete β-actin gene from Aedes albopictus. **Methods**: Total RNA was extracted from C6/36 cells. Degenerate primers were designed based on the β-actin sequences of An. gambiae, Ae. aegypti, Cx. pipiens pallens and D. melanogaster. By RT-PCR, the product was amplified, purified, cloned into the pGT vector and sequenced. The β-actin sequence was aligned and phylogenetically analyzed by the BLAST program and the CLUSTAL W program. **Results**: A sequence of 1132 bp including an open reading frame of 1131 bp was obtained (GenBank DQ657949). The deduced protein had 376 amino acids. Aligned to SWISS-PROT, it exhibited a high level of identity with β-actins from Anopheles, Drosophila and Culex at the amino acid sequence level. Phylogenetic analysis indicated that Ae. albopictus β-actin was much more homologous with invertebrate β-actin than with vertebrate β-actin. **Conclusion**: The gene may be used as the internal control in the experiments of Ae. albopictus.

Keywords: Aedes albopictus; β-actin; clone; nucleotide sequence; phylogeny

INTRODUCTION

Aedes albopictus is a maintenance (occasionally epidemic) vector of dengue viruses in parts of Asia and is a competent vector of several other viruses in experimental conditions. The C6/36 cell line, derived from Ae. albopictus cells, is widely used for propagation of dengue virus and flaviviruses[1-7].

Actin is one of the most abundant proteins in all eukaryotic cells and involved in a variety of processes such as muscle contraction, cell cytoskeleton, cell motility [8,9] and even parasite invasion into host cells [10]. The actin gene is conservative in diverse species, and its expression is constitutively and ubiquitously high in all tissues[11, 12]. Therefore, it has been widely used as an internal control in gene expression studies.

Although numerous actin genes have been identified from a variety of species, such as An. gambiae, D. melanogaster, M. musculus, H. sapiens, and S. cerevisiae, the actin genes from many mosquito species have not been found yet. We hereby report the isolation of the actin gene from Ae. albopictus. The features and applications of the predicted amino acid sequences are also discussed.

MATERIALS AND METHODS

Cell culture

The Ae. albopictus C6/36 cell line was obtained from China Center for Type Collection (CCTCC, China). The cells were maintained in Eagle's minimum essential media (EMEM) (Invitrogen, USA) supplemented with non-essential amino acids, fetal bovine serum (10%) (Sijiqing, China), penicillin and streptomycin. The cells were grown in a 5% CO_2 humidified incubator at 28℃.

RNA extraction and cDNA synthesis

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Total RNA was extracted from C6/36 cells with TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol, and contaminant genomic DNA was removed by DNase I treatment. cDNA was synthesized from 2 μg of total RNA with M-MLV reverse transcriptase (Promega, USA)and random oligonucleotide primers according to the manufacturer's protocol.

Cloning of Ae. albopictus β-actin gene and sequence analysis

Degenerate primers for the mosquito β-actin open reading frame(ORF) were designed based on β-actin sequences of An. gambiae, Ae. aegypti, Cx. pipiens pallens and D. melanogaster, and they were used to amplify a homologous region from Ae. albopictus C6/36 cell cDNA. The sequences of the oligonucleotide primers were forward: 5′-ATGTG(C/G/T)GACGA(A/C/T)GA(A/G/T)GTT-3′, reverse: 5′-CTTAGAAGCACTT (C/G)C (G/T)GTG-3′. PCR procedures were: initial denaturation at 94℃ for 5 min, followed by 30 cycles of 94℃ for 30 s, 58℃ for 40 s and 72℃ for 50 s with final 10 min extension at 72℃. The PCR product was separated by 1% agarose gel electrophoresis and purified using a QIA quick Gel extraction kit (Qiagen, Germany). Product was then cloned into the pGT vector (Tiangen, China) and sequenced.

Sequence alignment and phylogenetic analysis

The standard nucleotide-nucleotide and protein-protein BLAST sequence comparison (blastn and blastp; www.ncbi.nlm.nih.gov/BLAST/)and PSI-BLAST programs were used to search for sequences respectively in the GenBank and SWISS-PROT databases with similarities to the translated sequences of Ae. albopictus β-actin[13].Using the neighbour-joining method,sequence alignment and phylogenetic tree analysis were carried out by the CLUSTAL W program.

RESULTS

The isolated Ae. albopictus β-actin clone was 1132 bp long with an open reading frame of 1131 bp coding for a 376-amino acid protein (***Fig. 1*** and ***2***) and the sequence data was submitted to GenBank under accession No. DQ657949.

The deduced amino acid sequence of Ae. albopictus β-actin showed 99% identities with An. gambiae β-actin, 98% with Ae. aegypti β-actin and D. melanogaster β-actin and 90% identities with Cx. pipiens pallens β-actin (***Fig 3***). The predicted N-terminal sequences were similar to the corresponding

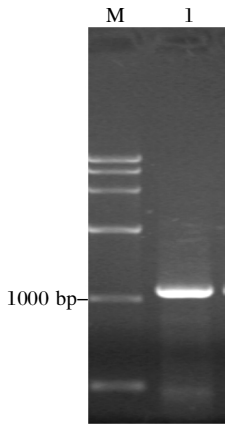

Fig 1 The PCR product of the complete β-actin sequence from Ae. albopictus. M: Marker; 1: Ae. albopictus β-actin

regions of β-actins from other invertebrates in that there were three acidic amino acids in the first five positions, a valine at position 11, a methionine at position 17 and a cysteine at position 18[14].

Based on some known actin gene sequences of An. gambiae, Ae. aegypti, Cx. pipiens pallens, D. melanogaster, C. elegans, M. musculus, H. sapiens, G. gallus, S. cerevisiae and Zebrafish, phylogenetic tree was constructed with the neighbor-joining method (***Fig 4***). It was indicated that Ae. albopictus β-actin was much more homologous with invertebrate β-actin than with vertebrate β-actin, except for Cx. pipiens pallens β-actin.

DISCUSSION

The complete β-actin gene from Ae. albopictus was obtained. The deduced amino acid sequence of Ae. albopictus β-actin showed a high level of sequence identity with other mosquito species, which was not surprising because of its evolutionary conservation as a housekeeping gene. It was not suitable for phylogenetic analysis to use amino acid sequence information in the case of the β-actin, because they showed too high level of sequence identities so that bootstrap support was lower than 50 % in many nodes [15]. This indicated that amino acid mutations were less tolerated in evolution than nucleotide mutations, which was also suggesting the importance of β-actin activities. Here we used nucleotide sequence data to construct the phylogenetic tree and the result showed that Ae. albopictus β-actin sequence was most closely related to An. gambiae β-actin.

The structural actins are a class of proteins represented by multiple subtypes with relatively distinct distributions [16]. They have demonstrated high conservation across genera. Because of their constitutive

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1 - ATGTGGGACGATGAGGTTGCCGCGCTCGTTGTCGACAATGGATCCGGTATGTGCAAGGCC - 60
    M  W  D  D  E  V  A  A  L  V  V  D  N  G  S  G  M  C  K  A
61 - GGTTTCGCCGGAGACGATGCCCCCCGTGCCGTCTTCCCGTCCATCGTCGGACGCCCCCGT - 120
    G  F  A  G  D  D  A  P  R  A  V  F  P  S  I  V  G  R  P  R
121 - CACCAGGGTGTGATGGTCGGTATGGGCCAGAAGGACTCGTACGTCGGCGATGAAGCCCAG - 180
    H  Q  G  V  M  V  G  M  G  Q  K  D  S  Y  V  G  D  E  A  Q
181 - AGCAAACGTGGTATCCTGACCCTGAAGTACCCGATCGAGCACGGTATCGTCACGAACTGG - 240
    S  K  R  G  I  L  T  L  K  Y  P  I  E  H  G  I  V  T  N  W
241 - GACGATATGGAGAAGATCTGGCATCACACATTCTACAATGAGCTGCGTGTCGCCCCGGAA - 300
    D  D  M  E  K  I  W  H  H  T  F  Y  N  E  L  R  V  A  P  E
301 - GAGCACCCAGTTCTCCTGACAGAGGCCCCCCTGAACCCAAAGGCTAACCGCGAGAAGATG - 360
    E  H  P  V  L  L  T  E  A  P  L  N  P  K  A  N  R  E  K  M
361 - ACACAGATCATGTTCGAAACCTTCAACACACCGGCCATGTACGTCGCCATCCAGGCCGTA - 420
    T  Q  I  M  F  E  T  F  N  T  P  A  M  Y  V  A  I  Q  A  V
421 - CTCTCGCTGTACGCTTCCGGTCGTACCACCGGTATCGTGCTCGATTCCGGAGACGGTGTC - 480
    L  S  L  Y  A  S  G  R  T  T  G  I  V  L  D  S  G  D  G  V
481 - TCCCACACAGTCCCCATCTACGAGGGTTATGCCCTGCCACACGCCATCCTGCGTCTGGAT - 540
    S  H  T  V  P  I  Y  E  G  Y  A  L  P  H  A  I  L  R  L  D
541 - TTGGCCGGTCGCGATCTGACCGATTATCTGATGAAGATCCTGACTGAACGTGGCTACTCG - 600
      L  A  G  R  D  L  T  D  Y  L  M  K  I  L  T  E  R  G  Y  S
601 - TTCACCACCACCGCCGAACGTGAAATCGTTCGTGACATCAAGGAAAAGCTGTGCTACGTC - 660
    F  T  T  T  A  E  R  E  I  V  R  D  I  K  E  K  L  C  Y  V
661 - GCCCTGGACTTCGAACAGGAAATGGCCACCGCTGCCTCGTCCTCCTCCCTCGAGAAGTCC - 720
    A  L  D  F  E  Q  E  M  A  T  A  A  S  S  S  S  L  E  K  S
721 - TACGAACTTCCCGACGGACAGGTCATCACCATCGGAAACGAACGTTTCCGTTGCCCAGAA - 780
    Y  E  L  P  D  G  Q  V  I  T  I  G  N  E  R  F  R  C  P  E
781 - GCCCTCTTCCAGCCGTCGTTCCTGGGTATGGAAGCCTGCGGTATCCACGAAACCACATAC - 840
    A  L  F  Q  P  S  F  L  G  M  E  A  C  G  I  H  E  T  T  Y
841 - AACTCGATCATGAAGTGCGACGTCGACATCCGGAAGGATCTGTACGCCAACACAGTATTA - 900
    N  S  I  M  K  C  D  V  D  I  R  K  D  L  Y  A  N  T  V  L
901 - TCCGGCGGTACCACCATGTACCCAGGAATCGCCGACCGTATGCAGAAGGAAATCACCGCC - 960
    S  G  G  T  T  M  Y  P  G  I  A  D  R  M  Q  K  E  I  T  A
961 - CTGGCCCCATCCACCATGAAGATCAAGATCATTGCCCCACCAGAGCGCAAATACTCCGTC - 1020
    L  A  P  S  T  M  K  I  K  I  I  A  P  P  E  R  K  Y  S  V
1021 - TGGATCGGTGGATCTATCCTGGCCTCGCTATCCACCTTCCAGCAGATGTGGATCTCCAAG - 1080
    W  I  G  G  S  I  L  A  S  L  S  T  F  Q  Q  M  W  I  S  K
1081 - CAGGAATACGACGAGTCCGGCCCATCCATTGTCCACAGGAAGTGCTTCTAAG - 1132
    Q  E  Y  D  E  S  G  P  S  I  V  H  R  K  C  F  *
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Fig 2 **The nucleotide and deduced amino acid sequences of Ae. albopictus β-actin (GenBank accession No. DQ657949). The deduced amino acid sequence was presented below the nucleotide sequence in single letter code. The asterisk indicated the stop codon.**

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A.albopictus      MWDDEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ  60
A.gambiae         MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ  60
D.melanogaster    MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ  60
A.aegypti         MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ  60
C.pipiens         MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ  60
                  * *:************************************************************
A.albopictus      SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM  120
A.gambiae         SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM  120
D.melanogaster    SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM  120
A.aegypti         SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM  120
C.pipiens         SKRGILTLMYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKASREKM  120
                  ******** ************************************************.****
A.albopictus      TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD  180
A.gambiae         TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD  180
D.melanogaster    TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD  180
A.aegypti         TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD  180
C.pipiens         TQVMFETFNTPAMYVAIQAVLSPYASGRTTGIVLDSGDGVSHTVPIYEGYAPQHAILRLD  180
                  **:****************** ************************** *******
A.albopictus      LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKS  240
A.gambiae         LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKS  240
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D.melanogaster   LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKS  240
A.aegypti        LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKS  240
C.pipiens        LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALGFEQEMATAASSSTLEKS  240
                 ************************************.***********:****
A.albopictus     YELPDGQVITIGNERFRCPEALFQPSFLGMEACGIHETT-YNSIMKCDVDIRKDLYANTV  299
A.gambiae        YELPDGQVITIGNERFRCPEALFQPSFLGMEACGIHETT-YNSIMKCDVDIRKDLYANTV  299
D.melanogaster   YELPDGQVITIGNERFRCPESLFQPSFLGMEACGIHETT-YNSIMKCDVDIRKDLYANTV  299
A.aegypti        YELPDGQVITIGNERFRCPEALFQPSFLGMEACGIHETT-YNSIMKCDVDIRKDLYANTV  299
C.pipiens        YELPDGQVITIDNERFRCPETLFRPSFIGMESAGVHEKTVFNSIKKCDVDIRKDLFANTV  300
                 **********.********:**:***:***:.*:**.* :*** **********:****
A.albopictus     LSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWIS  359
A.gambiae        LSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWIS  359
D.melanogaster   LSGGTTMYPGIADRMQKEITALAPSTMKIKIVAPPERKYSVWIGGSILASLSTFQQMWIS  359
A.aegypti        MSGGTTMYPGIADRMQKEITALAPSTIKIKIIAPPERKYSVWIGGSILASLSTFQQMWIS  359
C.pipiens        LSGGSTMFGGIADRMQRELTALAPPSIKVKIVAPPERKYSVWIGGSILASLSTFQXMWIS  360
                 :***:**: *******:*:*****.::*:**:********************** ****
A.albopictus     KQEYDESGPSIVHRKCF  376
A.gambiae        KQEYDESGPSIVHRKCF  376
D.melanogaster   KQEYDESGPSIVHRKCF  376
A.aegypti        KQEYDESGPGIVHRKCF  376
C.pipiens        KQEYDESGPGIVPRKCF  377
                 *********.** ****
```

Fig 3 **Amino acid sequence alignment of Ae. albopictus β-actin, A. gambiae β-actin, Ae. aegypti β-actin, Cx. pipiens pipiens β-actin, and D. melanogaster β-actin. Asterisks indicated identical amino acid and dots indicated similar amino acids.**

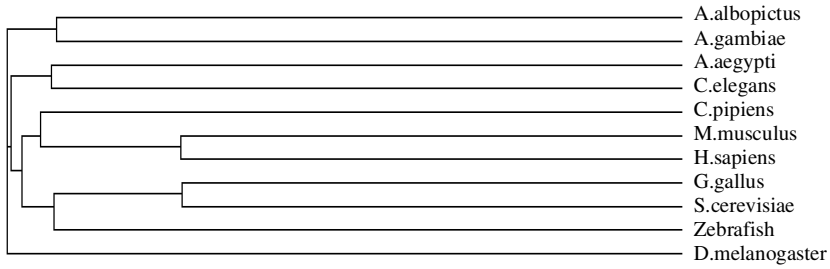

Fig 4 Phylogenetic relationships between Ae. albopictus β-actin and those of other species

and ubiquitous expression, they are frequently used as standards for expression studies at either RNA or protein level[17]. We have successfully used the β-actin genes as normalization factors for gene expression analysis[18,19]. Although Lin et al. reported that they successfully amplified one fragment β-actin gene from C6/36 cells[20], we could not get the same result by using the same primers.

In summary, we have identified the β-actin gene from Ae. albopictus. Based on the sequence analysis, the actin gene appears to be much conservative and it may be an appropriate candidate for use as a normalizing factor for characterizing gene expression in further studies.

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