



Molecular characterization of tumor necrosis factor receptor-associated factor 6 (TRAF6) in pearl oyster *Pinctada martensii*

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ABSTRACT. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a key signaling adaptor molecule for tumor necrosis factor receptor superfamily and Toll-like receptor/interleukin-1 receptor family members. It signals the upstream receptors and is involved in a wide range of biological functions, such as immunity and bone metabolism. In this report, the *TRAF6* gene from the pearl oyster *Pinctada martensii* (designated as *PmTRAF6*) was identified and characterized. The obtained full-length *PmTRAF6* cDNA was 2273 bp, containing a 5'-untranslated region (UTR) of 297 bp, a 3'-UTR of 128 bp with a 42-bp poly (A) tail, and an open reading frame of 1848 bp that encoded 616-amino acid residues. The deduced protein sequence of *PmTRAF6* contained a conserved TRAF family motif including a RING-type zinc finger, two TRAF-type zinc fingers, and a coiled-coil region followed by one meprin and TRAF homology domain. Multiple-sequence alignment indicated that TRAF6 was highly conserved among species, and *PmTRAF6* showed 53% sequence identity to *Azumapecten farreri* and *Mizuhopecten yessoensis*. Furthermore, an amino acid sequence containing a low-complexity region was inserted in the TRAF6s from mollusk. Quantitative real-time polymerase chain reaction analysis

demonstrated that Pm*TRAF6* was constitutively expressed in all tissues studied, with the most abundant mRNA expression in hepatopancreas and gill in *P. martensii*. After lipopolysaccharide stimulation, the expression of Pm*TRAF6* mRNA was dramatically upregulated. These results suggested that the obtained Pm*TRAF6* was a member of the TRAF6 family and perhaps involved in the innate immune response of pearl oyster.

Key words: TRAF6; Cloned gene; *Pinctada martensii*; Quantitative real-time reverse transcription-polymerase chain reaction

INTRODUCTION

Tumor necrosis factor receptor-associated factors (TRAFs), which function as adaptor proteins, are signal transducers for the tumor necrosis factor receptor (TNFR) family and Toll-like receptor (TLR)/interleukin-1 receptor (IL-1R) family members (Bradley and Pober, 2001; Wu and Arron, 2003; Kobayashi et al., 2004). Most of the TRAF family members contain a meprin and TRAF homology (MATH) domain in the C-terminal region and RING-type zinc fingers in the N-terminal region followed by a coiled-coil domain. The C-terminal MATH domain is essential for receptor binding. The N-terminal RING-type zinc fingers are crucial for the activation of the downstream signaling cascades (Lomaga et al., 1999; Ye et al., 2002; Wang et al., 2010; He et al., 2006, 2013).

In mammals, there are seven known TRAF family members (TRAF1-7) that play essential roles in signal transduction (Qiu et al., 2009; He et al., 2013). In both the gene structure and protein sequence homology of the C-terminal domain that is responsible for receptor binding, TRAF6 is the most divergent TRAF (Lomaga et al., 1999). With its special C-terminal domain, TRAF6 is the only TRAF that participates in the signaling pathways from the TLR/IL-1R family (Ye et al., 2002; He et al., 2006). TLR is a member of the pattern recognition receptors and could identify the pathogen-associated molecular patterns of different pathogens (He et al., 2013). IL-1 is crucial in the pro-inflammatory effects through the type-1 IL-1R (He et al., 2006). Although the extracellular domains of TLR and IL-1R are dissimilar, the cytoplasmic regions of each exhibit functional similarity and share a common motif, which is termed the TLR/IL-1R domain (Kobayashi et al., 2004). After activating TLR and IL-1R, a cytoplasmic adapter protein, MyD88, is recruited through their TLR/IL-1R domains, which is followed by the recruitment of IRAK Ser/Thr kinases, and then by association with TRAF6, which mediates the activation of downstream signaling via nuclear factor κ B (NF- κ B) and activator protein 1. With its capacity to signal a broad array of upstream receptors, TRAF6 has a spectrum of biological functions, such as innate and adaptive immunity, stress response, bone metabolism, and the lipopolysaccharide (LPS) signaling pathway (Lomaga et al., 1999). Recently, TRAF6 was reported to be involved in tumor formation and tumor angiogenesis through its function in the activation of NF- κ B and hypoxia-inducible factor-1 α (Starczynowski et al., 2011; Sun et al., 2013).

TRAF-6 may be a key molecule that participates in the innate defense system of mollusks (Qiu et al., 2009; He et al., 2013). The pearl oyster *Pinctada martensii* is the main species for marine pearl production in China and Japan. The identification and characterization

of TRAF6 in *P. martensii* will help our understanding of the innate immune response in pearl oyster. Although the *TRAF6* gene has been cloned and identified in several marine invertebrates, such as *Mizuhopecten yessoensis* (He et al., 2013) and *Azumapecten farreri* (Qiu et al., 2009), the *TRAF6* gene has not been characterized in detail and identified in *P. martensii*. To help our understanding of the innate immune response in pearl oyster, the aim of this research was to obtain the full-length *TRAF6* gene and to investigate the *TRAF6* gene expression patterns in different tissues and the temporal expression after LPS challenge in *P. martensii*.

MATERIAL AND METHODS

Pearl oyster and immune challenge

Pearl oysters, *P. martensii* (approximately 2 years of age) were obtained from Liushang, Zhanjiang, Guangdong Province, China. They were maintained at 25°-27°C in tanks with recirculating seawater for 1 week before the experiment. In the challenged group, 100 µL 10 µg/mL LPS (Sigma, USA) was injected into the adductor muscle of the pearl oysters. In the control group, 100 µL phosphate-buffered saline was injected in the same way. Hemocytes were collected from at least six pearl oysters 0, 2, 4, 8, 12, 24, and 36 h after injection, immersed in Trizol (Invitrogen, USA), and stored in liquid nitrogen.

Rapid amplification of cDNA ends (RACE)

5'/3'-RACE was applied to obtain the full-length cDNA of Pm*TRAF6* using the SMART RACE cDNA amplification kit (Clontech, Japan). Total RNA for the RACE reaction was extracted from the hemocytes of *P. martensii* using Trizol reagent. Gene-specific primers were designed based on the nucleotide sequences of the Pm*TRAF6* cDNA fragment obtained from the transcriptome of *P. martensii* (Zhao et al., 2012). Table 1 shows the polymerase chain reaction (PCR) primers used in this study.

Table 1. Primers for gene amplification and characterization.

Primer	Sequence	Application
β-actin-F	5'-TCCCTGGAGAAGAGC-3'	qRT-PCR
β-actin-R	5'-AGTTTCGTGGATGCC-3'	qRT-PCR
3'RACE-PmTRAF1	5'-ACGGGCATGTAATGGCTTTTTGTTTGG-3'	Inner PCR
3'RACE- PmTRAF 2	5'-TCACAGTCGTGTGGTCGATGACCAACGA-3'	Outer PCR
5'RACE- PmTRAF 1	5'-ATCCCTCATCAGACTTTGGTGTCCGAA-3'	Inner PCR
5'RACE- PmTRAF 2	5'-CAACATACTCTCCGACAACACGGCGTTA-3'	Outer PCR
PmTRAF-F	5'-GATGAAACGCTTGTAGCGA-3'	qRT-PCR
PmTRAF-R	5'-AGCACAGTCAAAGGGAGGAA-3'	qRT-PCR

DNA sequencing and bioinformatic analysis

The PCR products of the 5'- and 3'-ends were extracted, cloned into the pGEM-T easy vector (Promega), and sequenced. All the obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology

Information (NCBI; <http://www.ncbi.nlm.nih.gov/>). The open reading frame (ORF) was obtained using the ORF Finder tool (<http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi>). The molecular weight and theoretical isoelectric point were analyzed using a ProtParam tool (<http://web.expasy.org/cgi-bin/protparam/protparam>). The MEGA 4.0 software was used to construct the phylogenetic tree.

Tissue-specific expression and temporal expression of *PmTRAF6* after LPS stimulation

The adductor muscle, gill, mantle, hepatopancreas, gonad, and hemocytes were collected and immediately stored in liquid nitrogen. Total RNA was isolated using Trizol reagent according to manufacturer instructions. RNA integrity was analyzed by electrophoresis on 1.2% agarose gels. The RNA concentration was examined using a NanoDrop ND1000 spectrophotometer. The total RNA was reverse transcribed with oligo (dT)16 and M-MLV reverse transcriptase (Clontech). *PmTRAF6* mRNA expression was determined by quantitative real-time reverse transcription-PCR (qRT-PCR) with β -actin as an internal control (Jiao et al., 2012). Amplifications were conducted using SYBR green according to the manufacturer protocol. The qRT-PCR assay was performed in a C1000TM thermal cycler CFX96TM real-time system (Bio-Rad, USA).

Statistical analysis

The data from the experiments were analyzed using SPSS 13.0. P values less than 0.05 were considered to be statistically significant.

RESULTS

cDNA cloning, sequencing, and characterization of the *PmTRAF6* gene

Based on the unigene sequence, which was annotated as *TRAF6* in the transcriptome database of *P. martensii*, four *PmTRAF6* gene-specific primers were designed to amplify the 5'- and 3'-nucleotide sequences by 3'- and 5'-RACE technology. The PCR products were cloned and sequenced. Figure 1 shows that the complete sequence of the *PmTRAF6* cDNA was 2273 bp, containing a 5'-untranslated region (UTR) of 297 bp, an ORF of 1848 bp, and a 3'-UTR of 128 bp with a 42-bp poly (A) tail.

Characterization of the *PmTRAF6* deduced amino acid sequence

The deduced protein sequence of *PmTRAF6* was 616 amino acids with a mature molecular weight of 70.2 kDa and a theoretical isoelectric point of 6.42. The deduced amino acid sequence of *PmTRAF6* contained characteristic TRAF family motif predicted by the SMART program. One RING-type zinc finger was located between amino acids 60 and 98. There were two TRAF-type zinc fingers, which were found at amino acids 143-195 and 197-244. One coiled-coil region was located between amino acids 428 and 458, followed by one MATH domain that was predicted between amino acids 468 and 595 (Figure 1).

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1  acatggggggattccccactcigtctcaggacattaggaagttgaaagtaggaagtcgcacgaaggagatctctagttttagtggggtatctttttaaagctc
106  tatgtttaaantcttaactactggagaatagaactaccatcacagtacatctggataacaggtattaggaatagancagattgaaggtatgtaanaatgaaca
1  M A S N D N
211  ctgtactgaagacaagtctccaaatattgatatgacttagtactattgtgacaagatggaagttcagcacacagtttaatgactcATGCCATCGAATGATAAC
7  G G Q N D L Q S R E P H T R T S P P Q S S G T Y G S G S G D S F G H Q
316  GGAGGACAAAATGATTTACAGTCCAGAGAACCACATACCCGACTAGCCCGCTAAAGTTCOCCGACGTACGGATCAGGATCCGGAGATTCCTCGGACACCAA
42  S R D E G Y D L E F I L R D E K Y D C P I C L L V L R D P Y Q T Q C G
421  AGTCGTGATGAGGGATGATGTTGGAATTTATTTACGTGACGAAAAATGACTGTCTCTATTTGTTACTGGTTTTACGTGATCTTATCAAACTCAGTGTGGA
77  H R F C Q R C I F R W L R D S D Q R C P I D N A V L S E S M L F P D N
526  CATCGTTTCGGCAGCGGTGATCTTCAGATGGCTCAGGGATTCTGATCAGCGTTGCTCTATCGATAACGCCGTGTGTCGGAGAGTATGTTGCCGACAAAC
112  F A K R E I M G L Q V M C P N K K E G C N H I E T L K H L Q H H L D S
631  TTGCGAAGCGTGAATTTAGGGATTACAGGTCTATGTCCTAACAGAAGGAAGGTTGTAATCATATGAAACCCCTTAAGCATTACAGCATCATTAGATTA
147  C P P F K C E P C P N K C S H I L L R K D L K Q H L D H V C V K R K I Q
736  TGCCATTAAATGTGAACCTGTCTCTATAAATGCAGTCATATATTACTACGTAAGACCTTAAACAGCATCTTGATCATGTCGTGTGAACGAAAGATACAG
182  C H Q C P E Y I T A E Q L E D H L E K E C A M S M T E C P H C N A S M
841  GTGTCATAATGCCAGAGTATACAGCGGAACACTAGAGGATCACTTAGAGAAGGAATGCTATGCTATGACAGAGTGCACATGTAATGCTATG
217  M R E Q I K R H I D H D C M K V C I D C S F K K L G C T A S N I Q R G
946  ATCCGGGAACAGATTAAGAGGCATATAGACCATGATTGTATGAAGTTTGCATCGACTGTTCATTCAAGAAGCTAGGATGTACCCGATCAAAATCCAACTGGA
252  E M G K H R Q D Q I Q F H M N L M C R A L V V I T E K L N I T P D L L
1051  GAGATGGGAAACACAGACAGCAAAATCCATATGAACCTTATGTTAGAGCTCTGGTAGCATAAAGTGAACAACTGAACATAACACCCGATCTATTA
287  H P Q V G A T T S A A T A E I S Y N S C V N S L A D L Q R N L S E T L
1156  CACCCTCAAGTTGGTCCACAGCATCGCCGCCACTGCCGAAATCTTATAACTCCTGTGTAATAGCTTAGCAGATCTCCAAGCAATCTTCAGAACTCTC
322  A S L N L N N S P V V P T I S Q F L N Q P V S T I M T S P T V V S S T
1261  GCCAGTTTGAATTTGAATAATCACCGAGTGGCCACGATATCTCAGTTTCAACCAACCCGATCAACCATATGACCTCTCCCATGGTATCAACA
357  E S P R S H S R V V D D Q R L N S D Q T K V E S E V T Y T S V P S V D
1366  GAATCTCCGGGCTCACAGTCTGTTGGTGTGATGACCAAGCACTAAATAGTATCAAACTAAGTCGAGTCAAGTAACGTATACCTCAGTACCTCTGTGTGAC
392  Q E G A L R V P F D R L E F Q S M K D Q N A T Q D E S L A R H E H L I
1471  CAAGAGGGGGCGCTTCGAGTCCCATTGTATGCTTGAATTCAGTCCATGAAGATCAGAATGCTACGCAAGATGAAAGTTAGCAGACAGCAACACTTGATA
427  V E M K H K V E T S E K N S S I L L R R V R L L E N T L A E F E G R A
1576  GTAGAGTAGAAGCATAAGGTAGACGCTCAGAGAAAATAGCTCTATTTTATTAAGAAGGTGAGATTATGAAAAATACATTAGCCGAGTTTGAAGGACGGGA
462  C N G F F V W K V K N Y S K Y R R E A E L G E T T A I H S P A F Y S S
1681  TGTAAATGCTTTTGTGTTGAAAGTTAAAAATTAATTAATGCTGATGACCGGAGTTCAGGAGTACTGCCATCATAGCCCTGCGTTTACTCCAGT
497  C Y G Y K L C I R A N L N G V D A A R G T H L S I F I H F M Q G E F D
1786  TGTATGATATAAGTTATGATATCCGCCAAATTAATGCTGTTGATGCCGCAAGGGGAACCTATTTATCAATTTTCATTCATGCAAGGGGAATTTGAC
532  D I L D W P P S G R I M L T V M D Q N Q A M E L R H H V M E T L V A K
1891  GATATCTGGACTGGCCCTCAGTGGCCAAATATGTTGACCTCATGACCAAAAATCAAGCGATGGAATTACGTATCAGCTGATGGAACGCTTGTAGCGAAA
567  P N L A A F Q K P V T P R N H K G F G Y M E F L P L T V L D N S M Y I
1996  CCCAATGGCAGATCCAAAAGCTGTGACCCCGGAAATCAAAAAGCTTTGGCTATATGAAATTCCTCCCTTTGACTGTGCTAGACAATCTATGATATA
602  R N D T L I I K C H V I Q T N *
2101  AGAAATGATACCTCTTATTAAGTGTGATGTTATCAAACTTGAaccatgatgatgggttLtaatgtgataccagtaattgataaattgataaagata
2206  tatctgatatatgataaatttgtgaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

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Figure 1. Nucleotide and amino acid sequence of PmTRAF6. Numbers on the left indicate positions of the nucleotides in the PmTRAF6 cDNA sequence and the amino acid residues in the deduced protein. The sequence motif RING-type zinc finger (amino acids 60-98), two TRAF-type zinc fingers (amino acids 143-195 and 197-244), coiled-coil region (amino acids 428-458), and the MATH domain (amino acids 468-595) are underlined.

Homology analysis of PmTRAF6

The NCBI protein BLAST program was used to analyze the homology of the deduced amino acid sequence of PmTRAF6. PmTRAF6 had significant homology to a variety of TRAF6s that were previously submitted to the database. The sequence of PmTRAF6 showed 53% sequence identity to the TRAF6 proteins of *A. farreri* and *M. yessoensis*, 47% identity to that of *Euprymna scolopes*, and 32% identity to that of *Strongylocentrotus purpuratus*. Multiple-sequence alignment with other known TRAF6 proteins showed that most of the identities were located in the characteristic TRAF6 domains (Figure 2). These highly conserved domains indicated that the function of PmTRAF6 was similar to that of other identified TRAF6s.

Interestingly, similar to *A. farreri* (Qiu et al., 2009), additional amino acids were located between the second TRAF domain and the coiled-coil region was inserted in *P. martensii*, *M. yessoensis*, *E. scolopes*, and *S. purpuratus*.

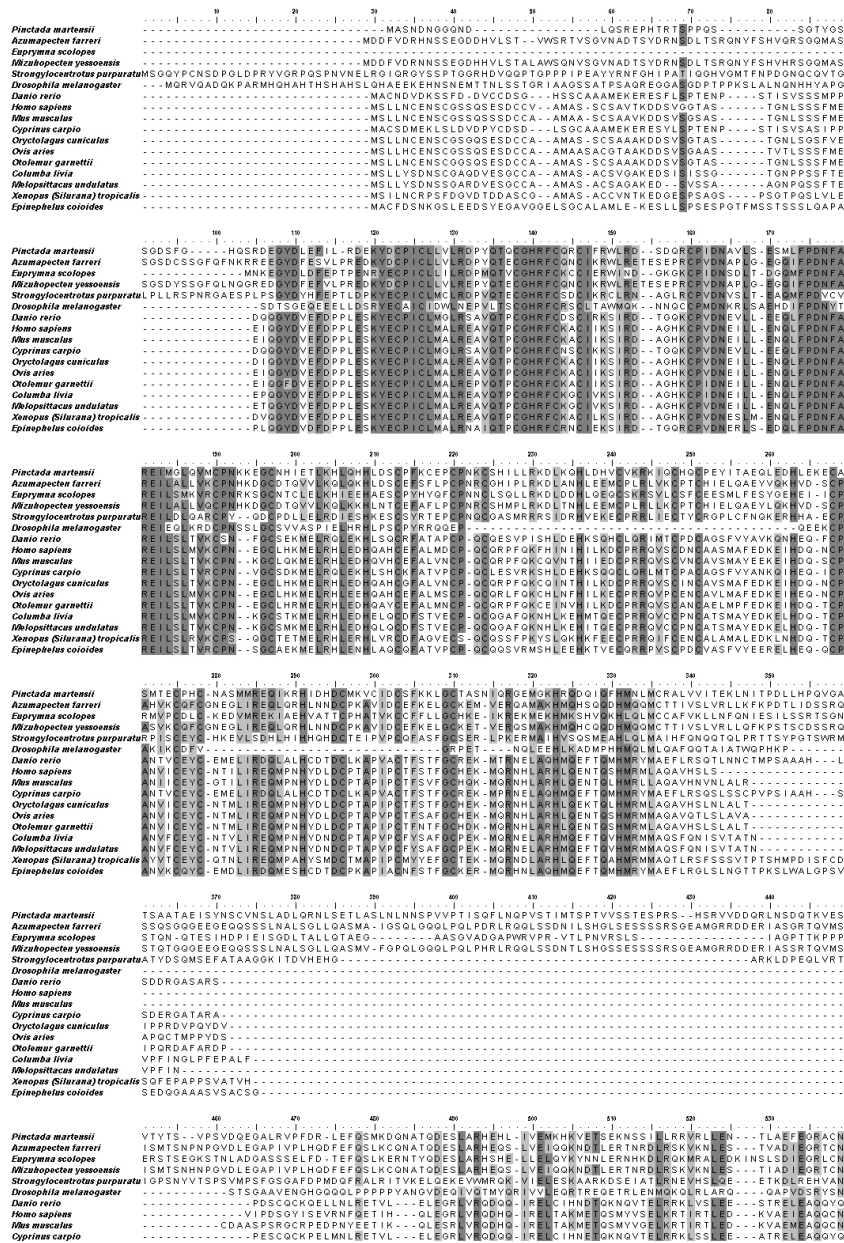


Figure 2. Alignment of the entire amino acid sequence of PmTRAF6 with other known TRAF6 sequences. Identical amino acids are highlighted in black and similar amino acids are highlighted in gray. The origin for each TRAF6 sequence is indicated on the left.

Further analysis of this insertion using the SMART software indicated that a low-complexity region (LCR) was contained in the TRAF6 proteins of mollusk, including *P. martensii*, *A. farreri*, and *M. yessoensis* (Figure 3).

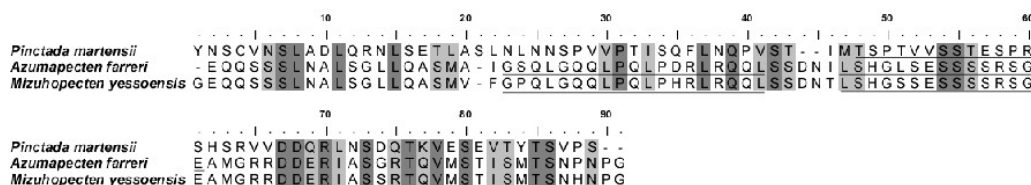


Figure 3. Analysis of the inserted amino acid sequence. The region containing the inserted sequence was analyzed using the SMART tool. The underlined sequences were predicted low-complexity regions.

In order to analyze the phylogenetic relationship between PmTRAF6 and other TRAF6s, a phylogenetic tree was constructed using the MEGA 5.0 software. The results indicated that *P. martensii* was clustered with TRAF6s from *A. farreri* and *M. yessoensis* (Figure 4).

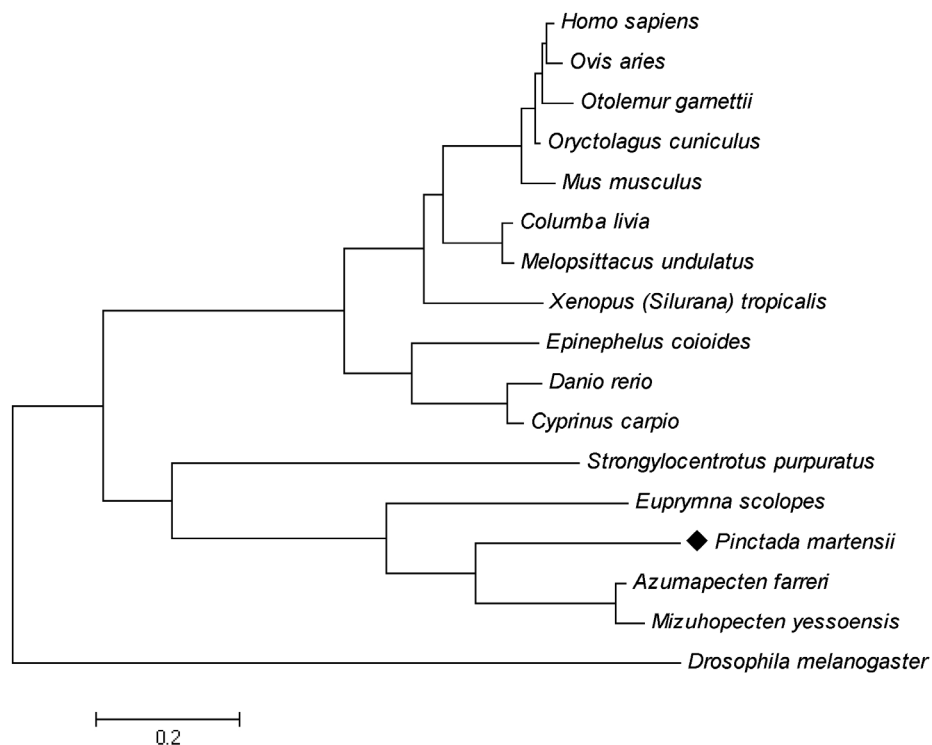


Figure 4. Phylogenetic tree showing the relationship between PmTRAF6 and TRAF6s from other species. Phylogenetic analysis was performed using the maximum likelihood method in MEGA 5.0 based on the sequence alignment results.

Quantitative analysis of *PmTRAF6* gene expression in different tissues

Next, qRT-PCR analysis was performed to determine the tissue-specific expression of *PmTRAF6* mRNA using β -actin expression as an internal control. *PmTRAF6* mRNA was constitutively expressed in all of the tissues detected (adductor muscle, gill, mantle, hepatopancreas, gonad, and hemocytes). The highest expression was observed in the hepatopancreas and gill, while a relatively lower expression level was detected in the hemocytes (Figure 5).

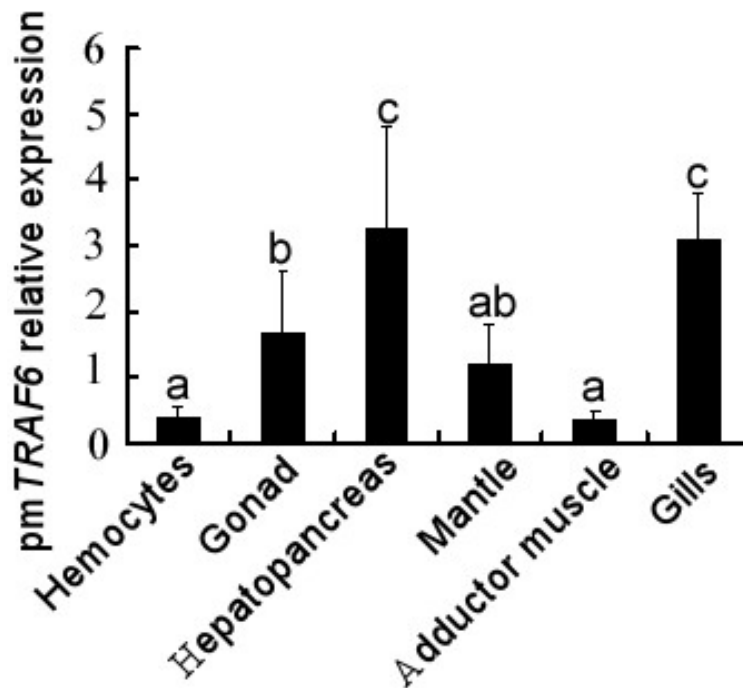


Figure 5. Expression patterns of *PmTRAF6* mRNA in different tissues. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was performed with RNA samples from the adductor muscle, gill, mantle, hepatopancreas, gonad, foot, and hemocytes. The pearl oyster β -actin gene was used as an internal control. Same letters above the bars represent no significant differences at the $P > 0.05$ level. Different letters above the bars represent significant differences at the $P < 0.05$ level.

Temporal expression pattern of *PmTRAF6* after LPS stimulation

To validate the function of *PmTRAF6* in the immune response, we analyzed the temporal expression of the *PmTRAF6* gene in hemocytes after LPS stimulation by qRT-PCR. The expression level of *PmTRAF6* was significantly increased 4 h after stimulation and was 4.02-fold higher than that of the control. Then, the expression level gradually decreased and returned to an expression level that was similar to that of the control 24 h after stimulation (Figure 6).

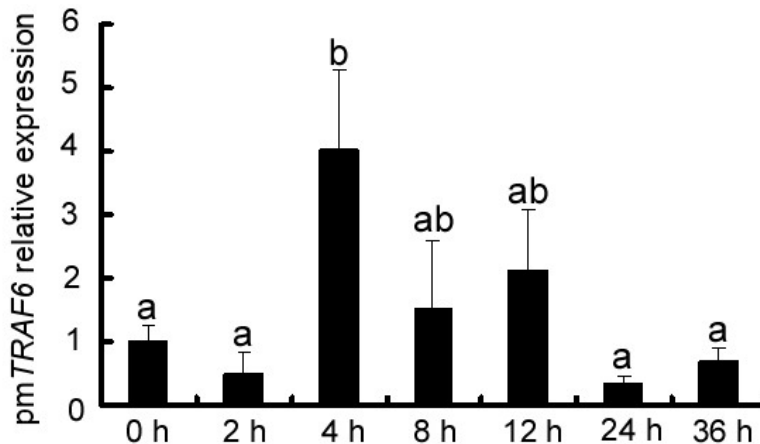


Figure 6. Expression patterns of Pm*TRAF6* mRNA after lipopolysaccharide (LPS) stimulation. qRT-PCR was performed using the RNA samples from hemocytes 0, 2, 4, 8, 12, 24, and 36 h after LPS stimulation. The pearl oyster β -actin gene was used as an internal control. Same letters above the bars represent no significant differences at the $P > 0.05$ level. Different letters above the bars represent significant differences at the $P < 0.05$ level.

DISCUSSION

The IL-1R/TLR superfamily plays critical roles in the innate immunity response to infection and injury (O'Neill, 2002). TRAF has been observed as the major signal transducer for the TNFR and IL-1R/TLR superfamilies. Additionally, numerous biological functions are mediated by TRAF through the induction of cell survival, proliferation, differentiation, and death (Kobayashi et al., 2004, 2009; Lu et al., 2013). TRAF6 is the only TRAF that is crucial for MyD88-dependent signaling pathways originating from the IL-1R/TLR superfamily. Thus, TRAF6 is functionally important in innate immunity. The first experimentally identified molluscan TRAF6 was obtained from *A. farreri* (CfTRAF6) (Qiu et al., 2009). CfTRAF6 mRNA expression was greatly inhibited by peptidoglycan, indicating its function in the immune response of scallop. Recently, He et al. (2013) obtained the full-length *TRAF6* cDNA from *M. yessoensis* using RACE technology and observed increased *TRAF6* mRNA expression after *Vibrio anguillarum* challenge. TRAF6 was also found in *Drosophila melanogaster* (DmTRAF6). DmTRAF6 was highly homologous to mammalian TRAF6 and was also identified as a downstream adaptor for TLR (Shen et al., 2001). These reports suggested that TRAF6 was highly conserved and functionally important in the innate immunity of organisms ranging from insect to mammal. To help our understanding of the innate immunity in pearl oyster, we obtained the *TRAF6* gene from *P. martensii* and identified its function in the innate immune response.

The full-length Pm*TRAF6* gene was 2541 bp and encoded 616 amino acids. The deduced protein sequence of PmTRAF6 shared a high degree of identity with other TRAF6s. Meanwhile, PmTRAF6 also contained the three conserved TRAF6 motifs, suggesting functional homology with other TRAF6s. Comparing TRAF6s from vertebrate and fruit fly revealed an insertion in the TRAF6 protein sequences of marine invertebrate, including *P. martensii*, *M. yessoensis*, *E. scolopes*, *S. purpuratus*, and *A. farreri*. Among these five TRAF6s, only the TRAF6s of mollusk were predicted to contain an LCR. LCRs in a protein are regions of biased composition, normally consisting of a regular repeat, cryptic repeat, and single-

amino acid repetitions. These LCRs could provide abundant material for new functions (Radó-Trilla and Albà, 2012). Additionally, these regions are not strongly conserved in length and evolve rapidly, although many participate in crucial molecular functions (Li and Kahveci, 2006). Some types of LCRs are usually found in proteins with particular functional classes, especially transcription factors and protein kinases (Faux et al., 2005). Compared with proteins without LCRs, proteins containing LCRs tend to have more interactions with other proteins (Faux et al., 2005). We proposed that TRAF6s in mollusk may have the ability to combine with other receptors and mediate other signal transductions, but this requires further experimental validation.

To explore the biological function of PmTRAF6 in *P. martensii*, tissue expression patterns were investigated using qRT-PCR. PmTRAF6 mRNA was highly expressed in the hepatopancreas and gills. The hepatopancreas of mollusks secretes various enzymes to hydrolyze microorganisms (Tiscar and Mosca, 2004). The molluscan gill is the main interface between aquatic organisms and the external environment and is considered as the first line of defense against bacterial infection (Lee et al., 2013). The higher expression of PmTRAF6 in these two organs suggested that it plays a role in the innate immune response.

The hemocytes of mollusk were believed to play crucial roles in the innate immune response. However, a relatively low expression level was observed in hemocytes, which was similar to the expression in hemocytes of *A. farreri* (Qiu et al., 2009). We speculated that the expression of PmTRAF6 in hemocytes may be induced in a stressed state. Then, the temporal expression of PmTRAF6 after LPS challenge in hemocytes was examined to determine if PmTRAF6 was involved in the innate immune response. LPS molecules are large and found in the outer membrane of Gram-negative bacteria. LPS functions as an endotoxin that elicits a strong immune response in animals (Kilár et al., 2013). In mammals, LPS responses are mediated by TLR4 (Chow et al., 1999). As the key signaling adaptor in the TLR signal pathway, TRAF6 could transmit the LPS signal to activate a transcription factor, NF- κ B, and other downstream kinases (Wong et al., 2004). In *P. martensii*, PmTRAF6 increased significantly in the first 4 h after LPS treatment, suggesting that PmTRAF6 is involved in LPS signaling and thus plays a role in the innate immune response of *P. martensii*.

CONCLUSION

In conclusion, using the partial sequence obtained from the transcriptome of *P. martensii*, we obtained the full-length PmTRAF6 cDNA and analyzed the characteristics of the ORF and the peptide sequence. The deduced protein sequence of PmTRAF6 contained a conserved TRAF family motif and was highly conserved among species. Furthermore, an LCR was inserted in the molluscan TRAF6s that was not present in the TRAF6 of other species. PmTRAF6 mRNA was highly expressed in the hepatopancreas and gill. After LPS stimulation, the expression of PmTRAF6 mRNA was significantly upregulated, suggesting that PmTRAF6 was involved in the innate immune response of pearl oyster. Further studies on the mechanism that controls the function of PmTRAF6 are necessary.

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