Cloning and expression of a transcription factor activator protein-1 member identified from the swimming crab *Portunus trituberculatus*

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Received: 12 June 2018 / Revised: 15 August 2018 / Accepted: 22 August 2018 / Published online: 25 September 2018
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Abstract

Transcription activator proteins are regulatory proteins that bind to the promoter regions of target genes. Transcription activator protein-1 (AP-1) regulates numerous genes related to the immune system, apoptosis, and proliferation. In this study, the full-length cDNA of AP-1 from *Portunus trituberculatus* (*PtAP-1*) was identified by expressed sequence tag analysis and cDNA-end rapid amplification. The gene is 1183 bp and encodes a 256-amino acid protein with a predicted molecular mass and isoelectric point of 28.96 kDa and 8.90, respectively. *PtAP-1* showed the highest expression level in the gonad tissue and the lowest expression level in blood, hemocyte, muscle, hepatopancreas, and gill, during the first 6 h of low-salinity stimulation (10%). Additionally, we observed steady decreases in *PtAP-1* mRNA expression in the gill, but at 12 h, expression was initially upregulated, followed by a significant decrease until restoration to baseline levels at 48 h. Additionally, *Vibrio alginolyticus* challenge resulted in significant upregulation of *PtAP-1* expression in the first 6 h, which was maintained at high levels for 48 h. From 48 to 72 h, we observed decreases in *PtAP-1* levels, although they remained significantly higher than those detected at baseline. These results suggested that *PtAP-1* is involved in the immune response and osmoregulation of crustaceans.

Keywords Transcription factor activator protein-1 · *Portunus trituberculatus* · Low salinity · *Vibrio alginolyticus* challenge

Introduction

Transcription factors are regulatory proteins that govern transcription inhibition or activation (De Zoysa et al. 2010) by binding to specific DNA sequences located in downstream promoter regions to control gene expression. Activating protein 1 (AP-1) is an important transcription factor and a member of a family of transcription factors that contain basic leucine zipper (bZIP) domains necessary for dimerization and DNA binding (Eychène et al. 2008; Schuermann et al. 1989). The major AP-1 subfamilies include Jun, Fos, and activating transcription factor proteins (Eychène et al. 2008; Karin and Liu 1997; Kamei et al. 1996).

Transcription factor AP-1 is a kind of chimeric protein of dimer complex, which consists of Jun (c-jun, JunB, JunD), Fos (c-fos, Fra-1, Fra-2, FosB), and atf protein, coded by proto-oncogenes jun, fos, and active transcription factor atf subunit gene, respectively, meanwhile, and AP-1 in working order in the form of homologous or heterologous Jun-Jun, Jun-Fos, and Jun-Atf (De Zoysa et al. 2010; Shaulian 2010; Vesely et al. 2009; Karin 2001; Halazonetis et al. 1988). This complex can bind to promoter and enhancer regions of target

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genes with high affinity, thereby regulating gene expression (Eriksson et al. 2010; Abate et al. 1991; Curran and Franza 1988). The broad spectrum of AP-1 proteins results in their binding affinity and specificity, as well as their regulation of multiple genes (Wagner and Eferl 2005; Curran and Franza 1988). Previous studies reported AP-1 involvement in wound healing, differentiation, cellular migration, immune responses, apoptosis, and inflammation (Wagner and Eferl 2005). Moreover, AP-1 as a regulator of cell life and death regulates gene expression in response to various stimuli, including stress conditions, growth factors, cytokines, and bacterial or viral infections (Zhou et al. 2018; Wu et al. 2015; Shaulian and Karin 2002). However, the molecular features and expression of AP-1 of crustaceans are seldom studied. The clone and expression profile analysis of AP-1 gene from Pacific white shrimp (*Litopenaeus vannamei*) has been carried out, indicating that AP-1 gene took part in shrimp innate immune response caused by WSSV (Wu et al. 2014). However, up to now, AP-1 has not yet been identified in *Portunus trituberculatus*.

The swimming crab (*Portunus trituberculatus*) is an important marine aquaculture species in China and yielded > 125,317 tons in 2016 (China fishery statistical yearbook 2017). However, disease, especially bacterial infections, has caused serious losses to the *P. trituberculatus* cultivation industry (Xia et al. 2018; Fu et al. 2016; Wang et al. 2007). Vibriosis is the main cause of high mortality of *P. trituberculatus*, especially in the summer when rain results in lower salinity in crab ponds (Ye et al. 2016). Without an acquired immune system, crabs defend against invading microorganisms solely with their innate immune system (Shentu et al. 2015; Wang et al. 2018). AP-1 is involved in the regulation of a variety of immune-related genes as a central transcription factor. Therefore, identification of crab AP-1 is important in order to improve the understanding of innate immune defense mechanisms in order to develop corresponding disease-control strategies. In this study, we cloned the full-length cDNA of *P. trituberculatus* AP-1 (*PtAP-1*) and evaluated its spatiotemporal-expression profile following exposure to bacterial challenge and low-salinity stress in order to clarify its immunoregulatory functions.

Methods

Experimental animals, bacterial challenge, and low-salinity stress

Crabs (*P. trituberculatus*; 151 ± 25 g) were purchased from a crab farm in Zhejiang Province, China, and cultured at between 17 and 19 °C in aerated tanks for 7 days prior to experiments. The crabs were fed razor clams and maintained in seawater (salinity 26‰); 50% of which was changed daily.

Under low-salinity stress conditions, 50 crabs were transferred to 10‰ salinity artificial seawater, and the gills of five crabs were randomly sampled at each of the following time points: 0, 3, 9, 12, 24, 36, and 48 h. Tissues were collected and stored at − 80 °C. The remaining 50 crabs in 26‰ salinity seawater were sampled at the same time points ($n = 5$ crabs/time point) as a control group for the reference sample.

For bacterial challenge experiments, *V. alginolyticus* was inoculated into marine broth 2216E at 28.5 °C and collected by 3000 g centrifugation for 10 min. The pellets were resuspended in seawater to 10^7 CFU/mL to induce significant immune responses without causing immediate mortality. Crabs ($n = 50$) were randomly sampled at 0, 3, 6, 12, 24, 48, and 72 h ($n = 5$ at each time point), and 1.5 mL of hemolymph was collected from each individual in the control and challenged groups. The hemolymph was centrifuged in order to collect hemocytes (2000 g for 10 min at 4 °C), and the RNA of the hemocyte pellets was extracted immediately. Hemocytes from crabs in the control group were used as reference samples.

RNA isolation and cDNA synthesis

An RNAiso Plus kit (TaKaRa, Dalian, China) was used to extract total RNA. The Promega M-MLV RT (Promega, Durham, NM, USA) was used for cDNA first-strand synthesis, and DNase I (Promega) was used to treat total RNA used as the template. Single-stranded cDNA was synthesized from total RNA (1 µg) in a final volume of 20 µL containing Tris-HCl (50 mM), random hexamers (50 pmol), RNasin (0.75 U), KCl (75 mM), DTT (50 mM), MgCl₂ (3 mM), dTTP, dGTP, dCTP, and dATP (0.2 mM, respectively), and M-MLV (200 U) reverse transcriptase (Promega). Reactions were performed at 37 °C for 1 h and terminated at 95 °C for 5 min. Products were stored at − 80 °C. A diluted cDNA mixture (1:50) was prepared for subsequent procedures.

Full-length cDNA cloning of *PtAP-1*

A *P. trituberculatus* cDNA library was constructed using the ZAP-cDNA synthesis kit and Gigapack III Gold cloning kit (Stratagene, San Diego, CA, USA). The expressed sequence tag (EST) sequences that were similar to known AP-1 sequences were identified by BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The 5' sequence of AP-1 was obtained from our preliminary transcriptome data, and the 3' ends of AP-1 cDNA were obtained by rapid amplification of cDNA ends (RACE) based on the EST sequence and using primers listed in Table 1. Nested PCR was used to clone the 3' end of *PtAP-1* using sense primers P1 and P2 according to the SMARTer RACE cDNA amplification kit (Clontech, Santa Clara, CA, USA) according to manufacturer protocol. The PCR temperature profile was 5 min at 94 °C and 32 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C, and 10 min at

Table 1 Gene-specific primers used in this study

Primer	Primer sequence (5'-3')	Sequence information
P1	CAGGAGGTTATGGATCATGTCA	Specific primer (for RACE)
P2	AGACACGAGGCACCTTTGAACT	Specific primer (for RACE)
P3 (forward)	CGCTACACTCCAACCTCCAGTA	Specific primer (real-time PCR)
P4 (reverse)	AACCTTATCCTCCAGACGGCT	Specific primer (real-time PCR)
β -actin (forward)	TCACACACTGTCCCCATCTACG	Reference gene
β -actin (reverse)	ACCACGCTCGGTCAGGATTTTC	Reference gene

β -actin was used as a reference gene (Lu et al. 2018)

72 °C. The PCR products were cloned into the pMD18-T simple vector (TaKaRa) and bidirectionally sequenced, and sequencing results were verified and subjected to cluster analysis.

Sequence and phylogenetic analyses

The cDNA sequence of AP-1 was analyzed by BLAST, and ExPASy (<http://www.expasy.org/>) was used to analyze the predicted amino acid sequence. SMART software (<http://smart.embl-heidelberg.de/>) was used to identify characteristic domains, and FASTA (Pearson 1990) was used to calculate the identity, similarity, and gap scores. Multiple sequence alignments of AP-1 were performed using the ClustalX program (<http://www.ebi.ac.uk/clustalw/>), and prediction of signal peptides was performed using SignalP 3.0 software (<http://www.cbs.dtu.dk/services/SignalP/>) (Thompson et al. 1994). The amino acid sequence of *PtAP-1* was analyzed by the neighbor-joining algorithm in Mega 3.1 (<http://www.megasoftware.net/>) to construct an unrooted phylogenetic tree.

Tissue distribution and *PtAP-1* expression in crabs following low-salinity stress and *V. alginolyticus* challenge

The total RNA of hemocytes, gill, muscle, hepatopancreas, and gonad was extracted from four randomly selected crabs. RNA was isolated, and cDNA was synthesized as described. The cDNA mixture was diluted 1:100 and stored at −80 °C.

AP-1-transcript tissue distribution and temporal-expression profiles in hemocytes challenged with *V. alginolyticus* and in the gill following exposure to low-salinity stress were determined by quantitative real-time PCR (qPCR). The cDNA product was amplified using *AP-1*-specific primers (Table 1), and the consequent PCR product was sequenced to examine the qPCR specificity. Relative *AP-1* expression level was analyzed using the comparative Ct method and according to the Ct of amplified *PtAP-1* and β -actin (internal control). Differences between the internal control (Δ Ct) and that for the target were used to normalize the differences in

qPCR efficiency and template amount. The sample Δ Ct was subtracted from the calibration Δ Ct value, resulting in $\Delta\Delta$ Ct. *PtAP-1* expression level was analyzed according to $2^{-\Delta\Delta\text{Ct}}$, with the results expressed as fold difference (Livak and Schmittgen 2001). *PtAP-1*-transcript tissue distribution was normalized to that in the muscle.

Statistical analysis

Data were analyzed using SPSS for Windows (v22.0; IBM Corp., Armonk, NY, USA). *PtAP-1* expression in different tissue and following *V. alginolyticus* challenge and low-salinity stress was compared using one-way analysis of variance, followed by Tukey's multiple-comparison post hoc test. Prior to analysis, raw data were assessed for normal distributions and variance homogeneity using the Kolmogorov–Smirnov test and Levene's test, respectively. Non-normal and heterogeneous data were transformed until normality and homogeneity were achieved. Differences were considered significant at $p < 0.05$.

Results

cDNA cloning and *PtAP-1* structure analysis

The complete *PtAP-1* cDNA sequence was 1183 bp and contained a 771-bp open reading frame encoding a 256-amino acid polypeptide with a predicted molecular mass of 28.96 kDa and a 8.90 isoelectric point (Fig. 1). SMART analysis revealed a characteristic Jun transcription factor domain (2–146 aa) and a bZIP region (177–241 aa) in the *PtAP-1* sequence (Fig. 1). The deduced amino acid sequence was highly homologous to those of previously identified AP-1s (Fig. 2). AlignX analysis showed that *PtAP-1* shared 59.2% identity with *Litopenaeus vannamei* (AIB53746), 58.7% with *Macrobrachium nipponense* (ASM46959), 53.4% with *Hyalella azteca* (XP_018022155), and 57.9% with *Penaeus monodon* (APL97141) (Fig. 2).

Atta cephalotes - - - - - mv r - - - s l t m e q t f y e d a s v y g a m n g r e n - s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 56
Trachymyrmex septentrionalis - - - - - mv r - - - s l t m e q t f y e d a s v y g a m n g r e n - s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 56
Atta colombica - - - - - mv r - - - m e q t f y e d a s v y g a m n g r e n - s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 50
Acromyrmex echinator - - - - - mv r - - - s l t m e q t f y e d a s i y g a m n g r e n - s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 56
Trachymyrmex cornetzi - - - - - mv r - - - s l t m e q t f y e d a g m y s a m n g r e n h s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 57
Wasmannia auropunctata - - - - - mv r - - - s l t m e q t f y e d a s i y t a m n g r e n - s v v q l k r n i t l d l n - g g c q r q g p q a k r p l g p 57
Cyphomyrmex costatus - - - - - mv r - - - n l t m e q t f y e d a s v y g a m n g r e n h s v v q l k r n i t l d l n - g c q r q g s q a k r p l g p 57
Vollenhovia emeryi - - - - - mv r - - - s l a m e q t f y e d a g v y g m g g r e n s v g q l k r n i t l d l n - g c q r q g p q a k r p l g p 57
Solenopsis invicta - - - - - mv r - - - s l t m e q t f y e d a g v y t a m n g - r d n t v v q l k r n i t l d l n - g c q r q g p q a k r p l g p 56
Pogonomyrmex barbatus - - - - - mv r - - - s m t m e p t f y e d a s v y n t m n g r e n - s m g q l k r n i t l d l n - g c q r q g s q a k r p l g p 56
Lasius niger - - - - - mv r - - - s l t m e p t f y e d a s i y g a m n g r e n - s m g q l k r n i t l d l n - g c q r q g p q a k r p l a p 56
Camponotus floridanus - - - - - mv r - - - s l t m e p t f y e n a s v y g t v n g r e n - s m g q l k r n i t l d l n - g c q r q g p q a k r p l g p 56
Oecocera biroi - - - - - mv l s q - - a t t m e p t f y e d a n v y - a m n g r e g - s m g q l k r n i t l d l n - g c q r q g p q a k r p l g p 57
Zootermopsis nevadensis m k s e q h d l l m v r n c v p p k m e t t f y e d h s f r l n g d v n - - - q l k r s m t l d l n - - - a s r g p - a k r p k f n - 61
Nilaparvata lugens - - - - - mv r n s s a s k m e p n f y e e g s f n q n v n - - - - - q l k r n i t l d l n - - - s a h n d a k r a f e c g 49
Litopenaeus vannamei - - - - - - - - - - - m e a t n y e d g s y n n y s k e s v n - - - - - q n k r k i t l d f t - - - - - r p s k k q - - - - 38
Penaeus monodon - - - - - - - - - - - m e a t n y e d g s y n n y s k e s v n - - - - - q n k r k i t l d f n - - - - - r p s k k q - - - - 38
Macrobrachium nipponense - - - - - - - - - - - m e a t n y e d g s y n t y q r e s l t - - - - - q n k r k i t l d l t - - - - - r p s k k q g - - - 39
Portunus triberculatus - - - - - - - - - - - n y e d g s y n n y t r e s v s - - - - - q i k r i t l d l t s - - - - - r t n k r q g t a 38
Hyalella azteca - - - - - - - - - - - m f m e t t n y e d t y k v y s k e n v a - - - - - q a k r k i r l d l n i p - - - - - g n g a k r q - - - 43

Atta cephalotes l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t s l p t p t t q i l f p k - - a v 111
Trachymyrmex septentrionalis l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t s l p t p t t q i l f p k - - a v 111
Atta colombica l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t s l p t p t t q i l f p k - - a v 105
Acromyrmex echinator l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t s l p t p t t q i l f p k - - a v 111
Trachymyrmex cornetzi l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t g l p t p t t q i l f p k - - a v 112
Wasmannia auropunctata l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m - p d g l v n - - - - - t g l p t p t t q i l f p k - - a v 111
Cyphomyrmex costatus l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t g l p t p t t q i l f p k - - a v 111
Vollenhovia emeryi l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q s d g l v - - - - - t s l p t p t t q i l f p k - - a v 112
Solenopsis invicta l p p a l n n v t p i l s s p d l n m l k i n s p e l e k l i m t q s d g l v - - - - - t s l p t p t t q i l f p k - - a v 111
Pogonomyrmex barbatus l p p v l n n a t p i l s s p d l n m l k i a s p e l e k l i m t q p d g l v - - - - - t s l p t p t t q i l f p k - - a v 111
Lasius niger l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q s d s l v - - - - - t g l p t p t t q i l f p k - - t v 111
Camponotus floridanus l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m t q s d s l v - - - - - t g l p t p t t q i l f p k - - t v 111
Oecocera biroi l p p a l n n v t p i l s s p d l n m l k i a s p e l e k l i m a q q d g l v - - - - - s g l p t p t t q i l f p k - - t v 112
Zootermopsis nevadensis - - - a v t a a p v l s s p d l n m l k i g s p e l e k l i i a q n g l v - - - - - t t t p t p - t q i l f p r - - t v 112
Nilaparvata lugens - - - - - a p v l s s p d l n h k l k i g s p d l e r l i s s g n - - - - - l p t p - t p s l f p r n p n a 92
Litopenaeus vannamei - - - p g g n f n p l l t s p d l n q l k i a s p e l e r l i m q q g s t - i l n s n - - - - - t v t t p s q f f f p s - k t a 92
Penaeus monodon - - - p g g n f n p l l t s p d l n q l k i a s p e l e r l i m q q g g - i l n s n - - - - - t v t t p s q f f f p s - k t a 91
Macrobrachium nipponense - - - t t g n f n p l l t s p d l n q l k i a s p e l e r l i i q n n s t - i l n s n - - - - - t v t t p s q f f f p s - k t t 91
Portunus triberculatus - - - t t t a n f n p l l t s p d l n q l k i a s p e l e r l i m q q s g t a i l g s g t a a i t a a p a t t t t t t t s q q f f f - k t t 105
Hyalella azteca - - - p a s l n p l l t s p d l n m l k i a s p e l e d l v l q q v g g - - - - - - - - - - - q h l f p - - - r v 83

Atta cephalotes e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Trachymyrmex septentrionalis e a q e l y a r g f v e a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Atta colombica e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 148
Acromyrmex echinator e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Trachymyrmex cornetzi e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 155
Wasmannia auropunctata e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Cyphomyrmex costatus e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m q - - - - - g a t y t t l e p p g s - - - - - 154
Vollenhovia emeryi e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 155
Solenopsis invicta e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Pogonomyrmex barbatus e a q e l y a r g f v d a l n e l h h s d s s q e p - g s v h - - - - - g a t y t t l e p p g s - - - - - 154
Lasius niger e a q e l y a r g f v d a l n e l h h s d s s q e p - g s m h - - - - - g a t y t t l e p p g s - - - - - 154
Camponotus floridanus e d q e l y a r g f i d a l n e l h h s d s s q e p - g s v h - - - - - g a t y t t l e p p g s - - - - - 154
Oecocera biroi e a q e l y a r g f v d a l n e l h h s d s s q e p - g s l h - - - - - g a t y t t l e p p g s - - - - - 155
Zootermopsis nevadensis e e q e m a r g f v d a l n e l h t s d s s q m s t i g g l p v d v p v t s n n i a g v g t y t t l e p h g q - - - - - 172
Nilaparvata lugens l e q e s v l g f v n a l n e n h q s d s s q a i q v e l p - - - - - r e s s s n s s a g s - - - - - 138
Litopenaeus vannamei t v e e e e a k g f e d t l e q l h h q d a i s t s g t v v v t s v a - - - p s v s s a p l q y t q l d v p i h q h h h a h p h q 158
Penaeus monodon t v e e e e a k g f e d t l e q l h h q d a i s t s g t v v v t s v d - - - p s v s s a p l q y t q l d v p i h q h h h a h p h q 157
Macrobrachium nipponense t l e q e e a k g f e d t l e q l h n q d g i a t s s g i v v t s v s d - a n p p v s s a p l q y t q l d v p t h - h h h p h l - q 157
Portunus triberculatus t l e e e e v k g f e d s l e q l h h q d s l h s n - - - - - l q y t q l e v p p t - - - - - 143
Hyalella azteca h l e q e e a k g f v d s l e q n h s q n n l s n h q i p h i i v t s n n - - - - - n t s q y t q l q p p q a - - - - - 134

Atta cephalotes - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Trachymyrmex septentrionalis - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Atta colombica - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 199
Acromyrmex echinator - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Trachymyrmex cornetzi - - - v q s t e s - - - - - - - t m s n p g - - - m v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 206
Wasmannia auropunctata - - - v q s t e s - - - - - - - t m s n p g - - - l l h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Cyphomyrmex costatus - - - v q s t e s - - - - - - - t m s n p g - - - l i h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Vollenhovia emeryi - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 206
Solenopsis invicta - - - v q s t e s - - - - - - - t m s n p g - - - l l h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Pogonomyrmex barbatus - - - v q s t e s - - - - - - - t l s n p g - - - l v q l k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Lasius niger - - - v q s t e s - - - - - - - t m s n p g - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Camponotus floridanus - - - v q s t e s - - - - - - - t m s n p n - - - l v h y k d e p q t v p s v s s t p p n s p i d m e n q e k i k l e r k r q 205
Oecocera biroi - - - v q s t e s - - - - - - - g i s n p a - - - l m h y k d e p q t v p s v t s t p p n s p i d m e n q e k i k l e r k r q 206
Zootermopsis nevadensis - - - v v i s q a n s f p p i s s a g y g i l s s p a p - - - a i m y k d e p q t v p s l g s t p p l s p i n m e c q e i k l e r k r q 236
Nilaparvata lugens - - - y g s m d p - - - - - t d f s t e l n - - - g v v k d e p q t v p n l g s - p p v s p i n m e c q e i k l e r k r q 190
Litopenaeus vannamei q v p v t s g a a - v s l s v s e s q h h v a a h l g l l p p a h i k e e p q t v p s v s g s p p l s p i d m e c q e i k l e r k r l 226
Penaeus monodon q v p v t s g a a - v s l s v s e s q h h v a a h l g l l p p a h i k e e p q t v p s v s g s p p l s p i d m e c q e i k l e r k r l 225
Macrobrachium nipponense q v p t s s a a p g v s l a v t - s e h h v s l g l l p h t s i k e e p q t v p n v s g s p l s p i d m e t q e i k l e r k r l 226
Portunus triberculatus - - - - - - - - - - - a a q n l i p p - - - h i k e e p q t v p s v g s p p l s p i n m e c q e i k l e r k r l 188
Hyalella azteca - - - - - - - - - - - q p f a t s n q - - - - h i k e e p l t v p s s - s p p l s p i n m e n q e i k l e r k r l 177

Atta cephalotes n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 273
Trachymyrmex septentrionalis n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 267
Atta colombica n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 273
Acromyrmex echinator n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 273
Trachymyrmex cornetzi n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 274
Wasmannia auropunctata n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s s i v h k l k e h v e r l k e q v m d h v h a g e q i m t v s n a q f 273
Cyphomyrmex costatus n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m t v n n a q f 273
Vollenhovia emeryi n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e p i m a v g n a q f 274
Solenopsis invicta n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e q i m a v n n a q f 273
Pogonomyrmex barbatus n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h a g e h i m t v n n h f 273
Lasius niger n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v n a g e h i m a g - - a f 271
Camponotus floridanus n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v n a g e h i v p g - - a f 271
Oecocera biroi n r v a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h s g c q m m s v a g a f 274
Zootermopsis nevadensis n r i a a s k e r r r k l e r i s r l e d k v k l l k g e n t e l s g v h k l k e h v e r l k e q v m d h v h s g c q i m v s - a h f 303
Nilaparvata lugens n r v a a s k e r r r k l e r i a k l e d k v r l l k g e n n e l s v v n k l k d q v e n l k q v l d h v q s g c a i m p a d q s f 258
Litopenaeus vannamei n r i a a s k e r r r k l e r i s r l e e k v k l l k g e n n e l q a v n k l r d q v e s l k e q v m e h v n s g c q i p f v t h q 293
Penaeus monodon n r i a a s k e r r r k l e r i s r l e e k v k l l k g e n n e l q a v n k l r d q v e s l k e q v m e h v n s g c q i p f v t h q 292
Macrobrachium nipponense n r i a a s k e r r r k l e r i g r l e e k v r i l k g e n a e l q g v n k l r d q v e s l k e q v m e h v n s g c q i p f v t h q 293
Portunus triberculatus n r i a a s k e r r r k l e r i s r l e d k v r i l k g e n v e l q n v n r l r d q v e s l k e q v m d h v n s g c q i p f v t h q 256

Fig. 3 Phylogenetic tree constructed using the neighbor-joining method. Common names and GenBank accession numbers are as follows: *Litopenaeus vannamei* (AIB53746), *Penaeus monodon* (APL97141), *Macrobrachium nipponense* (ASM46959), *Zootermopsis nevadensis* (XP_021915872), *Hyaella azteca* (XP_018022155), *Lasius niger* (KMQ96686), *Vollenhovia emeryi* (XP_011874216), *Trachymyrmex cornetzi* (XP_018363574), *Cyphomyrmex costatus* (XP_018394503.1), *Ooceraea biro* (XP_019886697), *Atta cephalotes* (XP_012058179),

Acromyrmex echinator (XP_011069320), *Atta colombica* (KYM80865), *Camponotus floridanus* (XP_011259614.1), *Trachymyrmex septentrionalis* (XP_018357058), *Pogonomyrmex barbatus* (XP_011639737), *Nilaparvata lugens* (XP_022197425), *Wasmannia auropunctata* (XP_011694334), and *Solenopsis invicta* (XP_011165845). Numbers next to the branches indicate bootstrap values for each internal branch in tree nodes from 1000 replicates

participates in ovary development (Tan 2012). Therefore, the abundance of *PtAP-1* transcripts in the gonad indicated its likely involvement in cell proliferation.

AP-1 is involved in differentiation, inflammation, apoptosis, wound healing, and cellular migration (Tuvikene et al. 2016). c-Jun N-terminal kinase, which regulates Jun protein activity and expression, also functions in response to environmental stress (Wagner and Nebreda 2009; Badshah et al. 2016). The optimal salinity level for *P. trituberculatus* is

Fig. 4 *PtAP-1*-transcript levels in hemocytes, muscle, hepatopancreas, gill, and gonad. Each symbol and vertical bar represent the mean $\pm$ standard error ($n=4$). Data designated by different letters showed significant differences ($P < 0.05$) among different tissues

25%, whereas salinity levels $< 10\%$ or $> 40\%$ represent stress conditions that negatively affect growth (Lu et al. 2012). In the present study, *PtAP-1* levels in the gill following low-salinity challenge were significantly upregulated at 12 h, showing a 2.5-fold increase relative to levels detected in controls ($P < 0.05$) (Fig. 5). These findings suggested that *PtAP-1*

Fig. 5 Temporal expression of *PtAP-1* in the gill after low-salinity challenge at 0, 3, 9, 12, 24, 36, and 48 h. *PtAP-1* mRNA level was normalized against the control group, with β -actin used as the internal control in order to calibrate cDNA-template levels for all samples. Each bar represents the mean $\pm$ standard error ($n=5$ individuals). Data for the same challenge time point with different letters indicate significant differences ($P < 0.05$) among different time points or groups

Fig. 6 Temporal expression of *PtAP-1* in hemocytes after *Vibrio alginolyticus* challenge at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h. Each bar represents the mean $\pm$ standard error ($n = 5$ individuals). Data for the same challenge time point with different letters indicate significant differences ($P < 0.05$) among different time points or groups

might participate in ion-transport processes by regulating the expression of related proteins.

AP-1 regulates the cell survival and apoptosis to protect organisms and activates the downstream immune responses (De et al. 2003). Activation of *PtAP-1* is necessary to initiate immune responses, whereas strict regulation of this activity is critical to prevent detrimental effects to the host resulting from prolonged AP-1 activation (Papoudou-Bai et al. 2016). In the present study, *PtAP-1* did not respond to bacterial infection during this period (Fig. 6). We speculate that the absence of a response might be because *PtAP-1* is a transcription factor rather than a pattern-recognition receptor for bacteria. The significant upregulation of *PtAP-1* expression at 6 h post-challenge indicated initiation of a response to bacterial infection. Subsequently, hemocytes produced by *PtAP-1* were recruited to augment the infiltrating hemocytes and prevent potential damage to other tissues, as well as to upregulate *PtAP-1* expression, which peaked from 6 to 48 h (Zhao et al. 2010). *PtAP-1* plays an important role in the immune response induced by bacterial challenge, during which hemocytes replicate rapidly, and the immune system is activated. Here, during the post-challenge stage, *PtAP-1* expression was downregulated, although these levels remained significantly higher than those detected at baseline (Fig. 6), suggesting that *PtAP-1* functions during later stages of infection. These results suggested the involvement of *PtAP-1* in the immune responses and osmoregulation of crustaceans.

Conclusion

In this study, we identified AP-1 from *P. trituberculatus*, with the structure of *PtAP-1* suggesting that it could be a new member of the AP-1 family. Our results showed that *PtAP-1* was most abundantly expressed in gonad tissue, functions in later stages of responses to bacterial infection, and might regulate proteins involved in ion-transport processes.

Acknowledgments We are grateful to the anonymous reviewers for their professional revision of the manuscript.

Authors' contributions C Wang and CK Mu conceived and designed the study. H Wang and MY Kong performed the cultivation of experimental animals. H Wang, C Shi, MY Kong, C Mu, and HL Wei performed and analyzed all the other experiments. H Wang and C Shi wrote the manuscript with support from all authors. All authors read and approved the final manuscript.

Funding This study was sponsored by the National Natural Science Foundation of China (41476124), China Postdoctoral Science Foundation (2018M632439), Ningbo university research fund (XYL18013), and K.C. Wong Magna Fund in Ningbo University.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate The animal subjects used in the present study are crabs, which are invertebrates and are exempt from this requirement.

Consent for publication Not applicable.

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