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Short communication

Molecular cloning and characterization of class I NF-κB transcription factor from pearl oyster (*Pinctada fucata*)

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ABSTRACT

NF-κB transcription factors play central roles in many important physiological and pathological processes including innate immune responses. Here we report the cloning of an NF-κB transcription factor, PfRelish from pearl oyster *Pinctada fucata*, one of the most important bivalve mollusks for seawater pearl production. PfRelish full-length cDNA is 3916 bp with an open reading frame of 3558 bp encoding a putative protein of 1186 amino acids. The deduced PfRelish contains a N-terminal RHD, a nucleus localization signal, an IκB-like domain with six ankyrin repeats and a death domain at the C-terminus, which is similar to class I NF-κB transcription factors. Comparison and phylogenetic analysis revealed that class I NF-κBs in mollusks including PfRelish might have most distant relationship to the arthropod Relish. Further expression analysis showed that PfRelish was apparently upregulated after *Vibrio alginolyticus* injection, which suggested that PfRelish was involved in the immune response to *V. alginolyticus*.

1. Introduction

Pearl oyster *Pinctada fucata* is distributed over the coastal area of South China and is one of the most important bivalve mollusks for seawater pearl production. Since the mid-1990s, disease outbreaks have resulted in mass mortality of pearl oyster and caused heavy economic losses [1]. In order to develop strategy and approaches to control diseases and enhance the pearl yields, many researches have focused on innate immune defense mechanism of pearl oyster, and some immune-relevant molecules such as IKK, IκB, F-type lectin, cathepsin L1 cysteine protease, multidomain galectin, heat shock protein 70, clip-domain serine protease, macrophage migration inhibitory factor like oxidoreductase, lipopolysaccharide and β-1, interferon-gamma-inducible lysosomal thiol reductase, 3-glucan-binding protein (LGBP) and interferon-gamma-inducible lysosomal thiol reductase have been identified [2–11].

NF-κB transcription factors play central roles in many physiological and pathological processes such as apoptosis, proliferation and differentiation, as well as in innate immune responses [12]. They have a well-conserved Rel homology domain (RHD) involved in DNA binding, dimerization and interaction with the inhibitor κB (IκB). When a cell receives signals, NF-κBs are released from the IκB, and rapidly enter the nucleus to activate expression of genes such as pro-inflammatory cytokines, adhesion molecules, chemokines and growth factors [12,13]. In mammals, five NF-κBs including NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel have been identified, and in *Drosophila melanogaster*, three NF-κBs, Dorsal, Dorsal-related immunity factor (Dif) and Relish have been demonstrated [14,15]. These NF-κBs were divided into class I and class II. Class I NF-κBs contain p105, p100 and Relish, which have long C-terminal domains inhibiting these NF-κB molecules. Class II NF-κBs consist of RelA, RelB, c-Rel, Dorsal and Dif, which contain C-terminal transactivation domains (TAD).

In aquatic invertebrate animals such as horseshoe crab *Carcinoscorpius rotundicauda* [16,17] and Pacific white shrimp *Litopenaeus vannamei* [18,19], many class I and II NF-κBs play archaic but fundamental roles in regulating the expression of critical immune defense molecules. In mollusks, class II NF-κBs have been identified from Pacific oyster *Crassostrea gigas* [20], abalones *Haliotis diversicolor supertexta* [21], sepiolid squid *Euprymna scolopes* [22] and pearl oyster *P. fucata* [23]. However, class I NF-κB was only characterized from freshwater snail, *Biomphalaria glabrata* [24]. Here we report the identification of class I NF-κB (named PfRelish) from pearl oyster, and showed its putative role in immune response to *Vibrio alginolyticus*.

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   1 gtttacaataaacggtgggttcaaaagatgggtttttccacatgttatatacgtacagaaccaatgctgggaatttttttgcaaatttccagaaatggtcaacg
   1                                        M   A   E   D   D   S   S   S   S   V   E   I   M   V   S   T   V   N   G   E   A   Y   N
 106 tttccggtatatatctgtaaacataaacatcaaataggATG GCA GAG GAT GAT TCT TCT TCT TCC TCT GTA GAA ATC ATG GTT TCC ACA GTA AAT GGA GAA GCT TAT A
  24 S K M D V S H M D D C D D D L Q P Y I A I V E Q P Q S R G F R F R Y E
 211 ATT CTA AGA TGG ATG TCA GCC ACA TGG ACG ATT GTG ATG ATG ATC TGC AGC CAT ACA TAG CCA TCG TAG AGC AAC CAC AGT CTC GAG GAT TTA GGT TCC GGT ATG
  59 C E G P S H G G L Q G E R S E K Y R R S F P S I K I Q N Y N G P A K I
 316 AGT GTG AAG GTC CAT CAC ATG GAG GAC TTC AGG GAG AAA GGA GTG AAA AGT ATA GGA GGT CAT TTC CAT CTA TCA AAT ACA GAA CTA TAA TGG GCC AGC CAA AA
  94 F V K L V T T E S I P R P H A H K L V G K N C Q D G V C V T E I K S G
 421 TTT TTG TGA AGC TGG TAA CAA CTG AAT CAA TAC CCA GAC CTC ATG CTC ATA AAC TTG TTG GAA AAA ATT GTC AAG ACG GAG TGT GTG TTA CGG AAA TTA AGT CAG
 129 N T V N F P N L C I Q H V T R R K A A D V I E Q R I H E S L K L D K L
 526 GAA ATA CAG TAA ATT TTC CCA ATC TCT GTA TAC AAC ATG TGA CAC GTA GGA AAG CAG CAG ATG TTA TAG AAC AGA GAA TCC ATG AGT CGT TAA AGC TGG ATA AAC
 164 V K M G D L N A E A D L T D D E K R Q A K E Q A V Q A A K D M Q L N V
 631 TAG TCA AAA TGG GTG ATC TTA ATG CTG AGG CTG ACT TAA CAG ATG ATG AGA AGA GAC AAG CCA AAG AGC AGG CAG TAC AAG CAG CCA AGG ACA TGC AGC TTA ATG
 199 V K L C F Q A Y L K D E N G T F S R L L T P V L S T A I Y D S K A P G
 736 TGG TCA AAC TAT GCT TCC AAG CTT ATC TCA AAG ACG AAA ACG GAA CAT TCA GTC GGC TTC TCA CAC CTG TTC TCT CTA CAG CAA TAT ACG ACA GCA AGG CAC CAG
 234 A N A L K I C R M D K Y G G C C S G G E E V F L L C E K V Q K D D I A
 841 GAG CCA ATG CAC TGA AGA TCT GCA GGA TGG ATA AGT ATG GAG GTT GCT GCT CTG GGG GTG AAG AGG TGT TCC TAC TCT GTG AAA AAG TAC AAA AAG ATG ACA TTG
 269 V R F V E Q D D D G N I S W E S F G N F G P F D V H R Q Y A I V F K T
 946 CTG TCC GAT TTG TAG AGC AGG ATG ACG ATG GAA ACA TTT CAT GGG AAT CGT TTG GGA ATT TTG GAC CTT TCG ATG TAC ATA GAC AGT ATG CTA TAG TAT TCA AGA
 304 P A Y H N Q R I S R P V N V T I M L Q R K S D Q E T S D P K S F T Y Y
1051 CGC CGG CAT ATC ACA ATC AGA GGA TCA GTC GTC CTG TGA ATG TTA CTA TAA TGC TGC AGC GCA AGT CTG ACC AGG AAA CTA GTG ATC CAA AGT CAT TTA CAT ATT
 339 P Q T F D K E E I E K K R K K P L P N Y P G G G G F T G G G E G G L H
1156 ACC CAC AAA CCT TTG ACA AAG AAG AGA TTG AGA AGA AGA GGA AGA AGC CCC TCC CTA ATT ATC CTG GAG GTG GGG GAT TTA CTG GAG GAG GAG AGG GGG GAC TTC
 374 G T P N G Q M N G T S S S F P P T Q G N L P G I I S E S E Q N L P V H
1261 ATG GCA CGC CTA ATG GTC AGA TGA ATG GAA CAT CTT CTT CCT TTC CAC CTA CCC AAG GCA ATC TGC CTG GTA TTA TAA GTG AGT CAG AAC AAA ATC TTC CTG TAC
 409 H S S H S S K A A N K R A K R T L K V D L E L D G G Q E A S V G I P A
1366 ATC ACA GCT CAC ATT CTT CTA AAG CAG CAA ACA AGA GGG CTA AAC GTA CGC TGA AAG TGG ACC TGG AAT TAG ATG GCG GAC AAG AAG CTA GTG TTG GCA TCC CTG
 444 T I S S I N S K P V S S F N F G A Q S I S G F P A F Q Q A G A Y I Q T
1471 CAA CAA TTT CTT CAA TAA ATT CCA AGC CTG TCT CTA GCT TTA ACT TTG GTG CAC AAT CTA TAT CTG GGT TCC CGG CAT TCC AGC AAG CAG GGG CTT ACA TAC AGA
 479 S G G P Q Y I M T Q Q G M V T G P P M D S Q F A F S N P T P Q T M Q Q
1576 CAT CTG GTG GCC CAC AGT ATA TAA TGA CCC AGC AGG GCA TGG TGA CTG GAC CCC CGA TGG ACT CAC AGT TTG CTT TCT CCA ATC CCA CAC CAC AAA CAA TGC AGC
 514 Q Q Q N F F N T Y S T Q T A G M Y S Q Q G H T Q G L L T Q G L L P R G
1681 AGC AAC AGC AGA ATT TCT TCA ATA CAT ACA GCA CAC AGA CCG CTG GGA TGT ACA GTC AAC AGG GCC ACA CCC AGG GTT TAC TCA CCC AGG GAC TAC TCC CCA GGG
 549 G A S S S K D K L L V S R G A L K E R N S N L D E L D S C Q V S S S G
1786 GTG GGG CCA GCT CCA GCA AAG ACA AAC TCT TGG TGT CTC GTG GGG CTT TGA AAG AGA GAA ACT CAA ATT TGG ATG AAT TAG ATT CTT GCC AAG TAT CAT CAT CAG
 584 Q S E M L V P R G V G N I D T K V N S R G H S T L L G D L C N G E E A
1891 GAC AGA GTG AAA TGC TGG TAC CAC GCG GTG TTG GAA ATA TTG ATA CAA AAG TGA ATT CAA GAG GCC ATT CTA CGT TAC TAG GAG ATT TAT GTA ATG GAG AGG AAG
 619 V P G T S E N D N K I K D T S L A G K E I S V D C D V S P T E R G T S
1996 CAG TAC CCG GTA CCT CAG AAA ATG ACA ATA AAA TCA AGG ATA CAT CTC TGG CAG GGA AGG AAA TCA GTG TAG ATT GTG ACG TTT CTC CGA CAG AGA GGG GGA CGA
 654 V E C S V H N R C D V E C Q T L V S H G D I Q E T S C Q T E T Q T S T
2101 GTG TAG AGT GTA GTG TAC ATA ACA GAT GTG ATG TCG AAT GTC AGA CAC TAG TGT CCC ATG GTG ATA TAC AGG AAA CTT CAT GCC AGA CGG AAA CTC AGA CAT CTA
 689 D Q N V R V C P V E V Q H V S V Q T E T D N V T K T A E R T V K A L Q
2206 CTG ATC AAA TGT GAG AGT TTG TCC TGT GGA AGT ACA ACA TGT CAG CGT GCA AAC AGA AAC GGA TAA TGT GAC TAA GAC AGC AGA GCG CAC AGT GAA AGC TTT AC
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