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First molluscan transcription factor activator protein-1 (Ap-1) member from disk abalone and its expression profiling against immune challenge and tissue injury

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ABSTRACT

The regulation of transcriptional activation is an essential and critical point in gene expression. In this study, we describe a novel transcription factor activator protein-1 (Ap-1) gene from disk abalone *Haliotis discus discus* (AbAp-1) for the first time in mollusk. It was identified by homology screening of an abalone normalized cDNA library. The cloned AbAp-1 consists of a 945 bp coding region that encodes a putative protein containing 315 amino acids. The AbAp-1 gene is composed of a characteristic Jun transcription factor domain and a highly conserved basic leucine zipper (bZIP) signature similar to known Ap-1 genes. The AbAp-1 shares 46, 43 and, 40% amino acid identities with fish (*Takifugu rubripes*), human and insect (*Ixodes scapularis*) Ap-1, respectively.

Quantitative real time RT-PCR analysis confirmed that AbAp-1 gene expression is constitutive in all selected tissues. AbAp-1 was upregulated in gills after bacteria (*Vibrio alginolyticus, Vibrio parahemolyticus* and *Lysteria monocytogenes*) challenge; and, upregulated in hemocytes and gills by viral hemorrhagic septicemia virus (VHSV) challenge. Shell damage and tissue injury also increased the transcriptional level of Ap-1 in mantle together with other transcription factors (NF-kB, LITAF) and pro-inflammatory TNF-α. All results considered, identification and gene expression data demonstrate that abalone Ap-1 is an important regulator in innate immune response against bacteria and virus, as well as in the inflammatory response during tissue injury. In addition, stimulation of Ap-1 under different external stimuli could be useful to understand the Ap-1 biology and its downstream target genes, especially in abalone-like mollusks.

1. Introduction

The ability to activate or induce cellular defense reactions in response to microbial challenge, inflammatory conditions, environmental stress or other immune modulation is a fundamental property of cells [1]. The transcriptional activation or control of the "on-and-off switch" is an essential and critical point in gene expression, which is mainly governed by transcription factors [2,3]. Transcription factors are a group of regulatory proteins that bind to specific DNA sequence in the promoter region of the downstream or target genes to regulate (either activate or suppress) gene expression at both transcriptional and posttranslational levels [4,5]. Ap-1 was one of the first transcription factors identified from mammals, and it involves a wide range of biological processes such as cell proliferation, survival, differentiation, growth, apoptosis, cell migration, inflammation, cellular migration, wound healing and transformation [6–9]. In mammals, the Ap-1 activator is a complex of oncogenic protein which is composed of Jun (c-Jun, JunB, and JunD), Fos (c-Fos, Fra-1, Fra-2 and FosB) and Atf (activating transcription factor) sub-families [7]. It consists of homo- or heterodimers of Jun–Jun, Jun–Fos, or Jun–Atf [8–10]. Moreover, Ap-1 is

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categorized in the class of basic leucine zipper (bZIP) transcription factors in mammals. Based on the numerous studies of mammalian AP-1 involved in signal transduction pathways, it was proposed that AP-1 can control selective gene expression in a variety of tissues or cell types under various stimuli [7].

Previous studies have suggested that Ap-1 protein function is associated with other transcription factors such as NF-kB [11] or Ets [12] to regulate the expression of a wide variety of genes, despite their difference regulatory mechanisms. As an example, Fujioka et al., [11] have reported an association between Ap-1 and NF-kB by showing that NF-kB regulated c-Fos and Ap-1 activity via expression of Elk-1. Furthermore, the stimulation of NF-kB increased the level of Elk-1 (a downstream gene of NF-kB). Elk and other associated genes can induce Fos and Ap-1 to a higher level, which then can activate the expression of Ap-1 downstream target genes. To date, Ap-1 is attracting much research interest since it has a wide range of interactions and involvement in various biological processes. Hence, investigating the diverse and complex interactions of Ap-1 in regulating immunity as well as inflammatory responses with respect to other transcription factors will be important to further understand the biological role(s) it plays.

Although molecular development and function of Ap-1 have been intensively studied in mammals, information available from fish and invertebrate species is limited. Fish Ap-1 or Jun family genes (components of Ap-1) have been identified from *Takifugu rubripes* (CAD56856), *Salmo salar* (ACN11435), *Oncorhynchus mykiss* (NP_001117883), and *Danio rerio* [13]. Invertebrate Ap-1 or Jun family members have been identified from *Drosophila melanogaster* (Ap-1) [14], *Ixodes scapularis* (Ap-1; XP_002404571), *Ciona intestinalis* (C-Jun; NP_001071996). The *Drosophila* Jun and Fos genes have been cloned by the screening of a *Drosophila* genomic library, and using a micro-sequencing technique of purified AP-1 protein followed by homology screening of cDNA libraries [15,16]. To our knowledge, there is no information relating to the molecular characterization and transcriptional regulation of Ap-1 in the phylum mollusk. In abalone, several immune functional genes have been cloned and characterized which are associated with pattern recognition [17] antiviral (Mx) [18], antigen processing (interferon gamma) inducible lysosomal thiol reductase –"GILT") [19], cytokine regulation (Suppressor of cytokine signaling 2-"SOCS-2" [20], inflammatory and apoptosis (TNF-α, Fas ligand) [21,22], antimicrobial response (defensin) [23], regulation of transcription (Rel family nuclear factor kappa B- "Rel/NF-kB" and lipopolysaccharide-induced TNF-α factor- "LITAF") [24] and inflammatory response (Allograft inflammatory factor-"AIF") [25]. The recognition of various immune genes and their similar homology to vertebrates provide clues that the abalone has diversified and complex immune system but regulatory mechanisms of those are largely unknown. Since Ap-1 as a central transcription factor link with various immune functional genes it provides researchers to understand largely unknown immune pathways in abalone. Additionally, in understanding the immune and inflammatory responses of the Ap-1, is also important to identify the Ap-1-inducible genes or associated molecules and their regulations which might contribute to the abalone host defense system.

The normalized cDNA library of disk abalone offers a unique opportunity to identify transcription factor Ap-1 in mollusk for the first time, and to elucidate its functional role and understand the evolutional relationships among the Ap-1 family members. Therefore, the goal of this study was to undertake the molecular characterization of the cDNA encoding transcription factor Ap-1 identified from disk abalone *Haliotis discus discus*. This study describes the regulation of Ap-1 after bacteria, VHSV challenge and tissue injury at transcriptional level. In addition, we compare the Ap-1 expression and transcriptional activation with other transcription factors such as NF-kB and LITAF and pro-inflammatory response TNF-α of disk abalone after tissue injury as a specific inflammatory stimulation.

2. Materials and methods

2.1. Identification and full-length cDNA sequencing of AbAp-1

We constructed a normalized cDNA library from disk abalone using the RNA isolated from whole tissues (gills, mantle, digestive tract, hepatopancreas, head, and muscle). The basic procedure of cDNA library construction, normalization and initial sequencing were described in a previous report [26]. Screening of abalone EST sequences helped to identify a partial sequence homologous to known Ap-1 sequences. The cloned gene was further sequenced to obtain full-length cDNA of abalone Ap-1 using the AbAp-1-IF internal primer (Table 1). Sequencing reactions were performed using a terminator reaction kit, Big Dye, and ABI 3700 sequencer (Macrogen, Korea).

2.2. Molecular characterization of AbAp-1

The Basic Local Alignment Tool (BLAST) program was used to search similar nucleotide and protein sequences to AbAp-1 [27]. Characteristic domains or motifs were identified using the PROSITE profile database [28]. Identity, similarity and gap percentages were calculated using FASTA program [29]. Pairwise and multiple sequence alignment were analyzed using the ClustalW version 1.8 program [30]. To conduct phylogenetic analysis, protein sequences were aligned with ClustalW. The tree was constructed using the MEGA 3.1 program by selecting 1000 bootstrap replicates of the Neighbor-joining method [31]. Presence of signal peptide was checked using the signal P prediction program [32].

Table 1
Description of primers used in the study.

Name	Accession number	Target	Primer sequence (5′–3′)
AbAp-1-IF	ACJ65689	Internal sequencing	AGGCAAGGGTGTGGTTTCTAGTCA
AbAp-1-F		Real time PCR amplification	ATCATTCAGGCAAACGGCATGGTC
AbAp-1-R		Real time PCR amplification	GAAGTTCGGCCAAAGCATCCACAA
AbNF-kB-F	GQ903763	Real time PCR amplification	AATGTTCTCCAGTGCTGCTCTGAC
AbNF-kB–R		Real time PCR amplification	AGCAGATCTTCCCTCACACTCGTA
AbLITAF-F	GQ903762	Real time PCR amplification	ACAGATCGTCACGGCAACTCACTA
AbLITAF-R		Real time PCR amplification	AAATCACACCCAACAAGGCACAGG
AbTNF-α-F	EF103427	Real time PCR amplification	TGAACAGAAAGGTGCAAGGCAACC
AbTNF-α-R		Real time PCR amplification	AAGAGTTGTCTCCCTGGTCCAACA
AbRibosomal-F	EF103427	Reference gene Real time PCR	GGGAAGTGTGGCGTGTCAAATACA
AbRibosomal-R		Reference gene Real time PCR	TCCCTTCTTGGCGTTCTTCCTCTT