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Molecular cloning and expression analysis of a fish specific interferon regulatory factor, IRF11, in orange spotted grouper, *Epinephelus coioides*

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ABSTRACT

Interferon regulatory factors (IRFs) are transcription mediators which play vital roles in multiple biological processes, such as antiviral defense, immune response, cell growth regulation and apoptosis. A fish specific IRF, termed IRF11, has been identified in previous study through searching fish genome databases. Herein, a transcript of IRF11, EcIRF11 was cloned from orange-spotted grouper, *Epinephelus coioides*. The EcIRF11 cDNA sequence has 1573 bp in length, encoding a putative protein of 261 amino acids, with a high degree of similarity found between EcIRF11 and its teleost counterparts. Comparative analyses in teleost genomes revealed that IRF11 may have an ancient origin at least 450 million years ago, and the locus harbouring IRF11 might have experienced chromosomal rearrangement and/or inversion during evolution. Expression analysis revealed that the other two members, IRF1 and IRF2 also in the IRF1 subgroup (SG) as IRF11, exhibited high expression levels in early experimental infection phase in response to viral stimulation of poly I:C and to bacterial stimulation of *Vibrio parahaemolyticus* infections in the fish, while EcIRF11 is not transcriptionally modulated at the examined time points except in kidney at 6 h following poly I:C stimulation. Taken together, the results obtained in this study indicate that IRF11 might have been originated from the same ancestor as IRF1 and IRF2, but exhibits distinct basal and induced expression, implying its different function which needs further characterization.

1. Introduction

Regulation of host antiviral response is a multifaceted and complex network [1,2], and interferon regulatory factors (IRFs) are a major group of transcriptional factors which regulate host immune responses at the crossroad of various signalling pathways to govern the expression of interferons (IFNs) and IFN-stimulated genes (ISGs) [3,4]. The IRF members are characterized with a well-conserved DNA binding domain (DBD) at the *N*-terminal region in which a signature tryptophan (W) pentad repeat is present [5,6]. The DBD region forms a helix-turn-helix motif with specific recognition of a DNA sequence termed as IFN stimulated response element (ISRE; consensus sequence: $^{A/G}$NGAAANNGAAACT) to regulate IFN transcription [7]. On the other hand, it has been revealed that IRFs can orchestrate various biological processes, such as cell growth regulation, differentiation and apoptosis [4,8–10].

IRF1, the first member found in the IRF family, was described initially in nuclear factor response to IFN-β expression [11]. Subsequently, the second member of this gene family which is structurally similar to IRF1, termed IRF2, was described with the ability to bind the same *cis* element as IRF1 and thereby suppressed the function of IRF1 [12]. The primary sequences of the two IRFs share a high degree of similarity in their *N*-terminal regions, but have a great diversity in their *C*-terminal regions in which acidic amino acids and serine/threonine residues are abundant in IRF1 but relatively high content of basic amino acids in IRF2, suggesting their distinct functions [12,13]. Ever since, a total of 10 IRFs, i.e. IRF1 to IRF10, have been identified in mammals, with the absence of IRF10 in human and mouse, birds, reptiles, and amphibians [14,15]. Four major IRF sub-groups (SGs) were identified in vertebrate taxa designated as IRF1-SG (including IRF1 and 2), IRF3-SG (IRF3 and 7),

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IRF4-SG (IRF4, 8, 9 and 10) and IRF5-SG (IRF5 and 6) based on molecular phylogenetic analysis [14,15]. In teleost, however, the constitution of IRF family is much more complicated than in higher vertebrates. For example, three IRF4 like genes were identified in zebrafish (*Danio rerio*), whilst only two IRF4 genes were observed in other teleost fish, such as in stickleback (*Gasterosteus aculeatus*), medaka (*Oryzias latipes*) and fugu (*Takifugu rubripes*). Moreover, an additional member in the IRF family, IRF11, was found only in fish with the same size of the first two exons as that of teleost IRF1 and IRF2 [15]. Interesting, IRF11 gene organization seems to be varied in fish. For example, the IRF11 genes of stickleback, medaka and fugu are consisted of three exons, while others in tetraodon and zebrafish have five and eight respectively [15]. Although the transcript of IRF11 has been characterized successfully in zebrafish and miiuy coraker (*Miichthys miiuy*) [16,17]. Information on the origin and evolution, as well as the immune role of IRF11 are still rather scarce.

Herein, a transcript encoding IRF11, named as EcIRF11 was cloned form orange-spotted grouper, *Epinephelus coioides*, an economically important marine aquaculture fish species in China and some southeast Asian countries [18]. The expression profile of EcIRF11, as well as the other two IRF1-SG members, EcIRF1 and EcIRF2, GenBank accession numbers, FJ376610.1 and FJ828966.1, were examined following polyinosinic:polycytidylic acid, poly I:C treatment and *Vibrio parahaemolyticus* infection. A systemic survey was also performed to examine the phyletic distribution of IRF11 genes in vertebrate, the position of IRF11 genes on teleost chromosomes was examined by using syntenic analyses. Furthermore, the origin and evolution of IRF11 were discussed in lineage of lobe and ray-finned fish.

2. Materials and methods

2.1. Fish and stimulation

Orange-spotted groupers (270–290 g in body weight) were maintained in aquarium with aeration at least two weeks for acclimatization prior to experiments. To analyze the constitutive expression level of EcIRF1, EcIRF2 and EcIRF11 *in vivo*, ten kinds of tissues/organs including liver, muscle, blood, skin, head kidney, kidney, spleen, thymus, gill and intestine were collected form eight healthy individuals and stored at −80 °C until use. To examine the temporal change in the expression of these three IRFs, all fish were challenged with poly I:C or *Vibrio parahaemolyticus* as previously described [19]. Briefly, three groups, each with eighteen groupers, were injected intraperitoneally with 200 μl poly I:C (dissolved in PBS, 2 mg/fish), or *V. parahaemolyticus* (1×10^6 colony forming units (cfu)/fish) or sterile PBS separately. At 6, 12, 24 h post-injection (hpi), six fish from each group were anaesthetized and sacrificed, tissue samples including head kidney, kidney, spleen, intestine, thymus, and gill were collected separately for the isolation of total RNA, as described in our previous study [19].

2.2. Cloning and sequencing of grouper IRF11 gene

Total RNA from spleen of grouper was extracted with Trizol (Invitrogen Corp) according to manufacturer's instruction, and the first stand cDNA was synthesized by using GoScript™ Reverse Transcription System (Promega). Degenerate primers (EcIRF11-dF and EcIRF11-dR, Table 1) were designed for amplifying EcIRF11 cDNA based on IRF11 sequences of *Danio rerio* (ENSDARG00000043492), *Gasterosteus aculeatus* (ENSGACG00000016299), *Oryzias latipes* (ENSORLG00000011716) and *Takifugu rubripes* (ENSTRUG00000000250). PCR product was inserted into pMD19-T vector (Promega) for being transformed into *Escherichia coli* strain DH5α, and sequencing of positive clones was performed in a commercial company in Nanjing, China. To obtain the remaining open reading frame and untranslated regions of the transcript, rapid amplification of cDNA end (RACE) PCR was performed based on the partial sequences by using GeneRacer Kit (Invitrogen), with subsequent RACE products sequenced as described above.

2.3. Bioinformatics analyses

The full length IRF11 cDNA sequences were translated to amino acid sequences using ExPASy translate tool (http://web.expasy.org/translate/). The deduced protein sequences were blasted against the NCBI non redundant protein database (nr) using BLASTP program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The protein domain features were predicted on the Pfam server (http://pfam.xfam.org/search). Sequence alignments were calculated using the CLUSTALW 1.8 program with manual adjustments. Sequence similarity analysis was performed on MatGAT 2.02 [20]. ProtTest was used to select the amino acid substitution model (http://darwin.uvigo.es/software/prottest2_server.html l). ML analyses based on the multiple protein alignment were performed using PhyML3.0 algorithm

Table 1
Primers used for sequencing and expression analyses in this study.

Primer name	Sequence of oligonucleotides (5′—3′)	Product size (bp)	Efficiency	Usage
Ec-IRF11-dF	GGMCGTGGCTGGARGMRCAGATTC	551	N/A	Internal region
Ec-IRF11-dR	CTTAAACACDGCYTCAKTCTGCTC		N/A	
Ec-IRF11-3Fout	AGCAACGCTTACAGAGTCTACAGGA	1188	N/A	3′ RACE
Ec-IRF11-3Fin	GGCAACCCTCAAGACCCATTTC	1055	N/A	
Ec-IRF11-5Rout	TGAAATGGGTCTTGAGGGTTGC	564	N/A	5′ RACE
Ec-IRF11-5Rin	GAAGAGTGTAGCATCTCGGTCAATA	267	N/A	
UPM	Long: CTAATACGACTCACTATAGGG CAAGCAGTGGTATCAACGCAGAGT		N/A	RACE
	Short: CTAATACGACTCACTATAGGGC		N/A	
NUP	AAGCAGTGGTATCAACGCAGAGT		N/A	
Ec-Actin_QF	GAGAGGGAAATCGTGCGTGA	195	1.981	Realtime PCR
Ec-Actin_QR	CATACCAAGGAAGGAAGGCTG			
Ec-IRF1_QF	TGAGCACAGCCCTGATTTCG	212	2.0154	
Ec-IRF1_QR	TGCCTGTGCCCAAAGTTATGT			
Ec-IRF2_QF	GCTGGGACCTGGAGAAAGATG	384	1.964	
Ec-IRF2_QR	GCTCCTGCTTGACCCAATCGT			
Ec-IRF11_QF	GGCTGGAGTATTGACCGAGATG	199	2.083	
Ec-IRF11_QR	TCCTGTAGACTCTGTAAGCGTTGC			

Note M = A + C, R = G + A, D = A + G + T, Y = C + T, K = G + T.