Molecular Cloning, Characterization and Expression Analysis of an ILF2 Homologue from *Tetraodon nigroviridis*

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Interleukin-2 enhancer binding factor 2 (ILF2) was reported to regulate transcription of interleukin-2 (IL-2), a central cytokine in the regulation of T-cell responses. This property of ILF2 was well characterized in human and mammals, but little is known in bony fish. In this paper, an ILF2 homologue was cloned and well characterized from *Tetraodon nigroviridis* for the further investigation of the function of ILF2 in bony fish. The full-length *Tetraodon* ILF2 cDNA was 1380 bp in size and contained an open reading frame (ORF) of 1164 bp that translates into a 387 amino-acid peptide with a molecular weight of 42.9 kDa, a 5' untranslated region (UTR) of 57 bp, and a 3' UTR of 159 bp containing a poly A tail. The deduced peptide of *Tetraodon* ILF2 shared an overall identity of 58%~93% with other known ILF2 sequences, and contained two N-glycosylation sites, two N-myristoylation sites, one RGD cell attachment sequence, six protein kinase C phosphorylation sites, one amino-terminal RGG-rich single-stranded RNA-binding domain, and a DZF zinc-finger nucleic acid binding domain, most of which were highly conserved through species compared. Constitutive expression of *Tetraodon* ILF2 was observed in all tissues examined, including gill, gut, head kidney, spleen, liver, brain and heart. The highest expression was detected in heart, followed by liver, head kidney and brain. Stimulation with LPS did not significantly alter the expression of *Tetraodon* ILF2. Gene organization analysis showed that the *Tetraodon* ILF2 gene have fifteen exons, one more than other known ILF2 genes in human and mouse. Genes up- and down-stream from the *Tetraodon* ILF2 were Rpa12, Peroxin-11b, Smad4, Snapap and Txnip homologue, which were different from that in human and mouse.

Keywords: Chromosome synteny, Cloning, Gene organization, Interleukin-2 enhancer binding factor 2, *in vivo* expression study, *Tetraodon nigroviridis*

Introduction

Nuclear factor of activated T cells (NF-AT) is a lymphoid-specific transcription factor that is thought to be largely responsible for determining the cell type-specific expression of the interleukin-2 (IL-2) gene. ILF2, also referred as NF45, is a component of the NFAT complex, as well as ILF3. ILF2 was originally copurified with ILF3 as a heterodimer, by virtue of its ability to bind to the NFAT binding site presenting on the interleukin-2 (IL-2) promoter, known as the antigen receptor response element 2 (ARRE-2), from the nuclear extract of stimulated Jurkat T-cell (Corthesy and Kao, 1994). Besides, ILF2 also has a potentiality to interact with RNA. It contains an amino-terminal RGG-rich single-stranded RNA binding domain and a DZF zinc-finger nucleic acid binding domain which is shared with ILF3 and other double-stranded RNA-binding proteins involved in gene regulation (Zhao *et al.*, 2005). ILF2 represents host cellular factors that can interact specifically with viral RNAs, including hepatitis B virus RNA (Shin *et al.*, 2002), bovine diarrheal virus RNA (Isken *et al.*, 2003) and adenovirus virus-associated RNAII (Liao *et al.*, 1998), as well as ILF3.

ILF2 contributes to gene regulation at levels of transcription, splicing and translation. It was reported that ILF3 functioned as both positive and negative regulator in gene expression, depending on the promoter context, while ILF2 acted as a regulator of ILF3 to stimulate its ability to activate gene expression (Reichman *et al.*, 2002). The function of ILF2 in up-regulating IL-2 expression has been recently demonstrated in mammals (Zhao *et al.*, 2005). ILF2 and ILF3 were also identified as component of spliceosome and participated in the regulation of RNA splicing (Zhou *et al.*, 2002). In addition, ILF2 may also function on regulating translation. As reported,

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ILF2 and ILF3 can interact with the dsRNA-dependent protein kinase (Langland *et al.*, 1999; Parker *et al.*, 2001), a putative regulator of translation initiation. ILF2 also can interact with translational elongation initiation factor 2 alpha, beta, and gamma subunits (Ting *et al.*, 1998). Besides, ILF2 can associate with RNAs in ribonucleoprotein complexes and participate in regulating of delayed translation of mRNAs (Curtis *et al.*, 1995).

ILF2 has been well characterized in mammals, however, little is known about its existence and biological function in bony fish. Fish, as a low vertebrate, is considered as an important evolutionary link between invertebrate and high vertebrate. The investigation of immune-related genes in fish can provide inspiration for elucidating the genesis and evolutionary progression of those genes. *Tetraodon nigroviridis*, as a genome sequenced model organism, provided a good model system for the study of cloning, identification and functional analysis of novel immune-related genes in fish. In this paper, we successfully cloned and characterized an immune-related gene ILF2 homologue from *Tetraodon nigroviridis*. The result of our research will pave the way for the further investigation on immunological function of ILF2 in low vertebrate and elucidating the composing and regulation mechanism of the IL-2 system. Besides, it will also provide some proof for investigation genesis and evolution of the IL-2 system.

Materials and Methods

Experimental fish. Green spotted pufferfish, *Tetraodon nigroviridis*, one year old of both sexes, weighing approximately 4–6 g, body length 4–5 cm, was obtained from the Institute of Fisheries of Zhejiang, China. The fish were kept in a recirculating water at 26°C, and fed with commercial pellets at a daily ration of 0.7% of their body weight. All fish were held in laboratory for at least two weeks prior to use in experiments to allow for acclimatization and evaluation of overall fish health. Only healthy fish, as determined by general appearance and level of activity, were used for studies.

Sequences retrieval. The *Tetraodon nigroviridis* genome database was searched by basic local alignment search tool analysis using human ILF2 amino acid sequence (Genbank accession no. NP_004506.2), and two stretches of sequences (chrUn_random:109879005,109882236; chrUn_random:109889297,109892638) sharing high homology were found. Subsequently, both sequences were retrieved and further analyzed using Genescan (Burge and Karlin, 1998), BLAST (Altschul *et al.*, 1990) and FASTA (Pearson and Lipman, 1988) programs. From this analysis, a possible coding sequence was found and was exploited to design primers for obtaining the full-length *Tetraodon* ILF2 cDNA.

RNA isolation and first strand cDNA synthesis. Healthy fish were sacrificed and total RNA was isolated from two main immune tissues, spleen and head kidney using TaKaRa RNAiso Reagent (TaKaRa), according to the manufacturer's instructions. The concentration of total RNA was measured by spectrophotometry,

Table 1. Oligonucleotide primers used to amplify cDNAs for *Tetraodon* ILF2 and β -actin

Primer name	Sequence (5'-3')
ILF2-F1	GTCCAGGGGTATCGTCCTTTTG
ILF2-F2	GTGGCTGCTCTGGGCAACAAG
ILF2-F3	GAGGAGGTGCGTCAGGTGG
ILF2-F4	GCTCCTACAAGAAGGGCACC
ILF2-R1	TGACGGTGTGGACCCTGAAGTTC
ILF2-R2	GACCAGAGACAAGATGGAGGACT
ILF2-R3	CCTCTTCAGCAGACACTCGC
3' race adaptor	AACTGCAAAAATAAGTCGCTC
5' race primer	p CATGGTGGCCCTTCTTG
β -actin-F	ACACCTTCTACAATGAGCTG
β -actin-R	CTGCTTGCTGATCCACATCT

and cDNA was synthesized from 1 μ g total RNA using TaKaRa 3' Full RACE Core Set (TaKaRa), according to the manufacturer's instructions and used as a template for gene cloning by PCR.

Cloning of ILF2 cDNA. PCR were performed according to standard protocols with the specific primers indicated in Table 1. Initially, PCR was performed using the cDNA prepared above with primers ILF2-F1 and ILF2-R1, which amplified part of the initial predicted sequence, to check that it was correct. The PCR conditions were as follows, 1 cycle of 94°C for 4 min and 30 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 10 min, and this PCR conditions were also employed in the following PCR reactions. Having isolated this partial sequence, the 3' end of *Tetraodon* ILF-2 was obtained by 3'-RACE, using TaKaRa 3' Full RACE core set. Semi-nest PCR approach was adopted to improve accuracy of PCR, in which primers ILF2-F1 and 3' race adaptor were used in first-round PCR and ILF2-F2 and 3' race adaptor were used in the second-round PCR. The 5' end was obtained by 5'-Full RACE Core Set (TaKaRa) according to the manufacturer's instructions. The cDNA used for 5' RACE was synthesized from 5 μ g total RNA isolated above, with a 5' end phosphorylated specific RT primer (5' race primer). The RNA in RNA/DNA hybrid was degraded by Rnase H. Then, the single-stranded cDNA was ethanol precipitated and ligated by T4 RNA ligase. Primers ILF2-F3 and ILF2-R2 were used in the first-round PCR, with ligation reactant used as a template and primers ILF2-F4 and ILF2-R3 were used in the second-round PCR.

The PCR products were purified from agarose gel using a Gel Extraction Kit (Qiagen) and ligated into pGEM-Teasy vector by T₄ DNA ligase following the manufacturer's instructions (Promega). Recombinants were identified through blue-white colour selection when grown on Ampicillin McConkey Agar plates (Sigma) and further checked by colony-PCR. Plasmid DNA from at least three independent clones was recovered using an alkaline lysis-based method (Birnbom, 1983) and sequenced on MegaBACE 1000 Sequencer (GE) using DYEnamic ET Dye Terminator Cycle Sequencing Kit (LPSrmacia).

Sequence analysis. Sequences generated were analyzed for similarity with other known sequences using the FASTA (Pearson

and Lipman, 1988) and BLAST (Altschul *et al.*, 1990) suite of programs. Multiple sequence alignment of all known ILF-2 amino acid sequences was performed using Clustal W (Thompson *et al.*, 1994), and the Phylogenetic tree was constructed from Clustal W-generated alignment. In addition, a series of analysis was done to characterize the deduced peptide of *Tetraodon* ILF2. The protein family signature was analyzed using the PROSITE database of protein families and domains (Bairoch *et al.*, 1997; Falquet *et al.*, 2002). Nuclear localization signal (NLS) of *Tetraodon* ILF2 was predicted using predictNLS program. Furthermore, subcellular location prediction was also done using LOctree (Nair and Rost, 2005) to characterize this peptide.

In vivo expression study. Fish were injected with 100 g LPS and left for 24 h before being sacrificed and healthy fish with no stimulation were used as negative control sample. Total RNA was extracted from various tissues of these fish, including gill, gut, head kidney, spleen, liver, heart and brain, using TaKaRa RNAiso Reagent (TaKaRa), according to the manufacturer's instructions. cDNA was synthesized from 1 µg total RNA, using TaKaRa RNA PCR Kit (AMV) Ver.3.0 (TaKaRa), and used as a template for PCR. The *Tetraodon* ILF2 expression was determined by semi-quantitative PCR with primers ILF2-F1 and ILF2-R1. Primers β-actin-F and β-actin-R derived from a *Tetraodon* β-actin cDNA sequence were used to confirm cDNA integrity and to normalize the cDNA concentrations to aid semi-quantitative analysis of *Tetraodon* ILF2 expression. PCR products were separated on 1.5% agarose gels and visualized by staining the gels in 100 ng/ml ethidium bromide (Sigma-Aldrich).

***Tetraodon* ILF2 gene organization and chromosome synteny analysis.** The *Tetraodon* ILF2 gene organization is elucidated using the *Tetraodon* ILF2 cDNA obtained by PCR and the *Tetraodon* genomic DNA sequence (chrUn_random:109879005, 109882236; chrUn_random:109889297, 109892638). The cDNA was aligned with the genomic DNA using GAP2 (Huang, 1994). Besides, an ILF2 homologue from *Fugu rubripe* was also found by searching *Fugu rubripe* genome database. The sequence of *Fugu* ILF2 (scaffold_291 238701-242630) was retrieved and the gene organization of *Fugu* ILF2 was analyzed subsequently. Genscan (Burge and Karlin, 1998), BLAST (Altschul *et al.*, 1990) and FASTA (Pearson and Lipman, 1988) programs were used to discover a number of genes up- and down-stream from *Tetraodon* ILF2, as well as *Fugu* ILF2. The order of these genes was compared with the corresponding locus at human chromosome 1q21.3 and mouse chromosome 3qF2.

Results and discussion

Cloning and characterization of *Tetraodon* ILF2 cDNA. Three overlapping products were obtained using RT-PCR with specific primers that were contained in the coding region, 5'UTR and 3'UTR of the *Tetraodon* ILF2 cDNA. The full-length *Tetraodon* ILF2 cDNA (Fig. 1, Genbank accession no. **DQ000647**) contains a 5' untranslated region of 57 bp, followed by an initiating ATG codon. The TGA termination

codon occurs at nucleotide 1219. Thus, the translation of the sequence from nucleotide 58 to 1218 codes for a 387 amino-acid peptide with a molecular weight of 42.9 kDa. The AATAAA consensus poly A signal is present at 128 nucleotides downstream from the stop codon. The poly A tail is 16 nucleotides downstream from the poly (A) signal sequence, which is in agreement with the fact that the signals are most often present at 11-30 nucleotides upstream from the poly (A) tail (Fitzgerald and Shenk, 1981).

PROSCAN and ScanProsite analysis showed that the deduced peptide of *Tetraodon* ILF2 contains two N-glycosylation sites (NETG, 160-163; NASQ, 214-217), suggesting that *Tetraodon* ILF2 may be a glycoprotein, two N-myristoylation sites (GGrFGs, 10-15; GImmTG 111-116) and a RGD Cell attachment sequence (RGD, 2-4). Besides, the peptide contains six protein kinase C phosphorylation sites (SyK, 107-109; TIR, 145-147; TvK, 172-174; TvK, 219-221; TaR, 313-315; SeK, 354-356), six Casein kinase II phosphorylation sites (TpaE, 67-70; TgfE, 162-165; SsaD, 167-170; SgfE, 235-238; StwD, 344-347; TpsE, 352-355) and a cAMP- and cGMP-dependent protein kinase phosphorylation site (KKqT, 109-112), and most of these sites are highly conserved through the species compared, suggesting that *Tetraodon* ILF2 may be substrate of protein kinases. Indeed, previous studies have shown that human ILF2 is a substrate for DNA-PK (Ting *et al.*, 1998) and PKR (Langland *et al.*, 1999) in vitro. Most of transcription factors, such as serum response factor, C/EBP, c-Jun and Oct-1, require phosphorylation for either transcriptional activation function, DNA binding, or a combination of both (Fimia *et al.*, 1998; Grenfell *et al.*, 1996; Kovacs *et al.*, 2003; Kumar *et al.*, 2003; Ortega-Perez *et al.*, 2005). However, whether *Tetraodon* ILF2 is phosphorylated by DNA-PK or PKR remains to be further elucidated.

PROSCAN and ScanProsite analysis also revealed that the *Tetraodon* ILF2 contains an amino-terminal RGG-rich single-stranded RNA-binding domain at positions 2-18 and a DZF zinc-finger nucleic acid binding domain that is shared with ILF3 and other double-stranded RNA-binding proteins involved in gene regulation, at position 98-318 (Fig. 1). Both domains have been reported to be present in mammalian ILF2 already (Zhao *et al.*, 2005), indicating that both domains are highly conserved through species. Subcellular localization prediction analysis showed that *Tetraodon* ILF2 is a nuclear, non-secreted protein. Consistent with mammals, none typical nuclear-localization signal was found in *Tetraodon* ILF2 by predictNLS. Previous studies showed that ILF2 was a nuclear factor and expressed predominantly in nucleus (Lopez-Fernandez *et al.*, 2002; Zhao *et al.*, 2005) while no NLS has been found in ILF2, which suggests a possibility of the presence of a novel NLS in ILF2.

The deduced amino acid sequence of *Tetraodon* ILF2 was aligned with other known ILF2 molecules, including human, mouse, zebrafish, xenopus, macaca, pongo, ciona, rat (Fig. 2), and the amino acid identity was 87, 87, 93, 86, 84, 87, 58 and 71%, respectively. *Tetraodon* and zebrafish ILF2 share 93%

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1      TGGCGATTGTCAGTTCTCGTAGAATCTTTACCTTCAACAATCAAAACGTCGTACACATG
61      AGAGGGGACCGCGGCAGGGGCGGGGCGGGCGCTTCGGGTCCCGAGGAGGACCGGTCCAG
      R G D R G R G R G G R F G S R G G P V Q
121     GGGTATCGTCCTTTTGTTCCTCACATCCCTTTGATTTTACGTGTGTGAGATGGCCCTTT
      G Y R P F V P H I P F D F Y V C E M A F
181     CCCAGAGTCAAACCCCGCGGACGAGACGGCCTTCAGCGAGTGTCTGCTGAAAGAGGAAC
      P R V K P P P D E T A F S E C L L K R N
241     CAGGACCTGAGCCCAACGCCTGCAGAGCAGTCTCCATCTTGTCTCTGGTCACCAAGATC
      Q D L S P T P A E Q S S I L S L V T K I
301     AATAATGTGATCGACAACCTGATCGTTGCCCGGGAACCTTTGAAGTGCAAATCGAGGAG
      N N V I D N L I V A P G N F E V Q I E E
361     GTGCGTCAGGTGGGCTCCTACAAGAAGGGCACCATGATGACGGGCCACAACGTGGCCGAC
      V R Q V G S Y K K G T M M T G H N V A D
421     CTGGTGGTATCCTCAAGATCCTCCCAACCTGGAGGCGGTGGCTGCTCTGGGCAACAAG
      L V V I L K I L P T L E A V A A L G N K
481     GTGGTGGAGACCTGCGCACGCAAGACCCCAAGAAGTCCTGTCCATGCTGACCAACGAG
      V V E T L R T Q D P T E V L S M L T N E
541     ACCGGCTTTGATGATCAGCTCAGCCGACGCCACCGTCAAGATCCTCATCACCGTCCCG
      T G F E I S S A D A T V K I L I T T V P
601     CCCAACCTGCGCAAGCTAGACCCCGAGCTGCACCTGGACATCAAGGTTCTTCAGAAGCCT
      P N L R K L D P E L H L D I K V L Q N A
661     CTGCGCGCATCCGTCACGCCCGCTGGTTTGAAGAGAACGCCCTCCAGTCCACGGTCAAG
      L A A I R H A R W F E E N A S Q S T V K
721     GTTCTGATCCGCTGCTGAAGGACCTGCGGCAGCGCTTCTCGGGCTTCGAACCCCTGACC
      V L I R L L K D L R Q R F S G F E P L T
781     CGCTGGATCCTGGACCTGCTGGGCCACCATGCTGTGATGAACAACCCGTCACGACGCCG
      R W I L D L L G H H A V M N N P S R Q P
841     CTGGCCCTGAACGTGGCCTACAGGCGCTGCCTGCAGATGTTGGCTGCTGGACTCTTCCTG
      L A L N V A Y R R C L Q M L A A G L F L
901     CCAGGATCGGTGGGCATCAGGACCCCTGTGAGAGCGGGAACCTTCAGGGTCCACCGTTC
      P G S V G I T D P C E S G N F R V H T V
961     ATGACCCCTGGAGCAGCAGGACATGGTGTGCTTACCGCCCGGACCCCTGGTGAGGGTTCTG
      M T L E Q Q D M V C F T A R T L V R V L
1021    TCCACGGAGGCTTACGGAAGATCCTCGGCCTGGAAGGAGAGCTAGCTACCTGGTGTCA
      S H G G F R K I L G L E G D A S Y L V S
1081    GAAATGTCCACGTGGGACGGCGTGATCGTCACGCCCTCGGAGAGGCCCTACGAGAAGCCT
      E M S T W D G V I V T P S E K A Y E K P
1141    CCTGAGAGGAAGGAGGAGGAGGAGGCTCTGGAGGAGGAGGAGACGGCGAGGACGAC
      P E R K E E E E E A L E E G G D G E D D
1201    GGCATGGAGACGCAGGACTGAGCCCAAGGAGCAGTCGCCTGCCCTCAGCCCCGCCCCAGC
      G H E T Q D *
1261    TGGCCCTCAGCCCCGCCCTGGCTCTGGCCCCGCCCAACCTGCCTTCGATTGGTTTGT
1321    GTCACCTTTGGTTTCGTTGTATCAGCTGAAATAAAATTGTGTTTATGTTCCAAAAA

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Fig. 1. Compiled full-length *Tetraodon* ILF2 cDNA sequence. The start and stop codon, the poly(A)⁺ signal (AATAAA) and the poly(A) tail in the 3' UTR are in boldface. The RGG-rich single-stranded RNA-binding domain (amino acids 2-18) and DZF zinc-finger nucleic acid binding domain (amino acids 98-318) are underlined.

identity, and both are 387 amino acids in size, 3 amino acids shorter than human and mouse ILF2. Besides, human and Pongo ILF2 are totally identical, human and mouse ILF2 show 97% identity, with the exception of a single conservative substitution of I for V at position 142 of the mouse sequence (Lopez-Fernandez *et al.*, 2002; Zhao *et al.*, 2005). The high conservation suggests an important role for ILF2 during evolution. The phylogenetic tree shown in Fig. 3 indicated that the newly isolated *Tetraodon* ILF2 could be classified with the group of zebrafish, mouse, Pongo and human.

In vivo expression study. *Tetraodon* ILF2 was constitutively expressed in all tissues of healthy fish, including gill, gut, head kidney, spleen, liver, brain and heart, and the highest expression was found in heart, followed by liver, head kidney and brain. Stimulation with LPS did not significantly alter the expression of *Tetraodon* ILF2 (Fig. 4). The ubiquitous expression of ILF2 is consistent with a postulated role in gene regulation at the levels of transcription and RNA splicing (Saunders *et al.*, 2001; Scherl *et al.*, 2002; Zhou *et al.*, 2002).

***Tetraodon* ILF2 gene organization and chromosome synteny analysis.** By comparing the full-length *Tetraodon* ILF2 cDNA with the corresponding genomic sequence, the organization of *Tetraodon* ILF2 gene was elucidated (Fig. 5). In the genomic sequence, the intron splicing consensus (GT/CAG or GT/AG) is conserved at the 5' and 3' ends of the introns. The two copies of *Tetraodon* ILF2 genes are identical, with the exception of a few differences in introns (Fig. 5, Fig. 6). Interestingly, the *Tetraodon* ILF2 gene was found to have fifteen exons, one more than other known ILF2 genes in human and mouse (Fig. 6). The similar gene organization was also found in *Fugu* ILF2. Compared *Tetraodon* ILF2 with human and mouse ILF2, the combination of fourteenth exon and fifteenth exon was corresponding to the fifteenth exon of human and mouse ILF2. This consistency with *Tetraodon* ILF2 may well suggest that the original ILF2 gene in the evolutionary level of fish may consist of fifteen exons, but when evolved into higher vertebrates, such as mammals, the fourteenth intron disappeared and the adjacent two exons (the fourteenth and fifteenth exons in fish) were combined into one

Fig. 2. Multiple alignment of the predicted *Tetraodon* ILF2 amino acid sequence with those of known ILF2. The GenBank accession numbers of the ILF2 genes are Human, NP_004506.2; Mouse, NP_080650.1; Tetraodon, AAY18083.1; Zebrafish, NP_998401.1; Rat, Q7TP98; Ciona, BAE06575.1; Pongo, Q5RFJ1; Xenopus, Q6P8G1; Macaca, BAE01639.1.

Fig. 3. Phylogenetic tree showing the evolutionary relationship of *Tetraodon* ILF2 with other proteins of the ILF2 family. The estimated genetic distance between sequences is proportional to the length of the horizontal lines, which connect one sequence to another. Numbers at nodes indicate the bootstrap percentages from the neighbor-joining analysis. GenBank accession numbers for these amino acid sequences are as follows: Human, NP_004506.2; Mouse, NP_080650.1; *Tetraodon*, AAY18083.1; Zebrafish, NP_998401.1; Rat, Q7TP98; Ciona, BAE06575.1; Pongo, Q5RFJ1; Xenopus, Q6P8G1; Macaca, BAE01639.1.

Fig. 4. Analysis of the tissue expression of *Tetraodon* ILF2. A. RT-PCR was performed using primers specific for *Tetraodon* β -actin and ILF2 and cDNA from a variety of tissues (gill, gut, head kidney, spleen, liver, heart and brain) from both healthy control fish and fish injected with 100 μ g LPS. B. The *Tetraodon* ILF2 mRNA levels are expressed as a ratio to β -actin mRNA levels after densitometric scanning of the gels stained with ethidium bromide. The mean expression level $\pm$ S.D was calculated from three healthy control fish and three LPS-stimulated fish for each of the seven tissues tested. One-tailed t-test showed no significantly change in expression in those tissues.

(Altschul *et al.*, 1990) and FASTA (Pearson and Lipman, 1988) suite of programs, genes up- and down-stream from the *Tetraodon* ILF2 were discovered (Fig. 7). The gene upstream from *Tetraodon* ILF2 is a Rpa12 homologue, followed by

ILF2, Peroxin-11b, Smad4, Snapap and Txnip, while the genes upstream from *Fugu* ILF2 is Mgat1, followed by ILF2, Peroxin-11b, Smad4, Snapap and Txnip, the genes upstream from human ILF2 is Npr1, followed by ILF2, Snapap, C1orf77, S100a1, S100a13 and S100a14, and the genes upstream from mouse ILF2 is Npr1, LOC628292, followed by ILF2, Snapap, 2500003M10Rik, S100a1, S100a13 and S100a14. The chromosome synteny around *Tetraodon* ILF2 is similar to that of *Fugu*'s but different from that of human and mouse's, indicating the possibility that chromosome synteny around ILF2 is conserved through fish but is different from high vertebrate.

ILF2 was reported to participate in regulating the expression of IL-2, which is a central cytokine in the regulation of T-cell responses (Smith, 1988) and controls the amplification of naive T cells (Waldmann *et al.*, 2001). Transgenic Jurkat T-cells that stably overexpress ILF2 sense cDNA demonstrated a 120-fold enhancement of IL-2 reporter gene activation and a 2-fold enhancement of endogenous IL-2 gene expression as compared to the control (Zhao *et al.*, 2005). Recently, a first conclusive evidence for the existence of interleukin 2 in bony fish was provided (Bird *et al.*, 2005) which demonstrated the existence and importance of IL-2 system in fish during evolution. As a pivotal regulatory component in IL-2 system, whether ILF2 participates in the regulation of IL-2 expression and other immune-related responses in bony fish is interesting to be elucidated.

In conclusion, an ILF2 homologue was cloned and characterized in *Tetraodon nigroviridis*. This work will pave the way for the further investigation into the biological function of ILF2 gene in fish as well as the genesis and evolution of IL-2 immune system.

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