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Genomic organization, gene duplication, and expression analysis of interleukin-1β in channel catfish (*Ictalurus punctatus*)

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Abstract

Interleukin-1β (IL-1β) is one of the pivotal early response pro-inflammatory cytokines that enables organisms to respond to infection and induces a cascade of reactions leading to inflammation. In spite of its importance and two decades of studies in the mammalian species, genes encoding IL-1β were not identified from non-mammalian species until recently. Recent research, particularly with genomic approaches, has led to sequencing of IL-1β from many species. Clinical studies also suggested IL-1β as an immunoregulatory molecule potentially useful for enhancing vaccination. However, no IL-1β genes have been identified from channel catfish, the primary aquaculture species from the United States. In this study, we identified two distinct cDNAs encoding catfish IL-1β. Their encoding genes were identified, sequenced, and characterized. The catfish IL-1β genes were assigned to bacterial artificial chromosome (BAC) clones. Genomic studies indicated that the IL-1β genes were tandemly duplicated on the same chromosome. Phylogenetic analysis of various IL-1β genes indicated the possibility of recent species-specific gene duplications in channel catfish, and perhaps also in swine and carp. Expression analysis indicated that both IL-1β genes were expressed, but exhibited distinct expression profiles in various catfish tissues, and after bacterial infection with *Edwardsiella ictaluri*.

Keywords: Interleukin; IL-1; Inflammation; Infection; Fish; Catfish; Gene; Immunity

1. Introduction

Immunity, the state of protection from disease, has both non-specific and specific components. Immunologists have long been interested in the components of the innate (non-specific) immune system and their interactions with the better-characterized adaptive immune system (for reviews on innate immune components and their functions, see Philpott and Girardin, 2004; Gasque, 2004; Khalturin et al., 2004; Bohana-Kashtan et al., 2004; Bottino et al., 2004; Beutler, 2004). One particular molecule, interleukin-1β, has important functions in both the innate and adaptive immunities. Interleukin-1 (IL-1) is one of the pivotal early response pro-inflammatory cytokines that enables organisms to respond to infection and induces a cascade of reactions leading to inflammation. Many of these reactions are mediated through regulating expression of other cytokines (for a recent review, see Bird et al., 2002; Huising et al., 2004). The IL-1 gene family consists of 10 ligand proteins in mammals including IL-α (IL-1F1), IL-β (IL-1F2), IL-1 receptor antagonist (IL-1ra, IL-1F3), IL-18 (IL-1F4), and 6 new members identified recently through the analysis of expressed sequence tags (EST) referred to as IL-1F5 through IL-1F10 (Smith et al., 2000; Debets et al., 2001). While the biological functions of the six new members (IL-1F5-IL-1F10) await elucidation, the functions of the other four of the IL-1 family proteins are well studied in mammals. IL-1α and IL-1β share a common receptor on target cells and therefore share many biological effects, but IL-1β is more potent in activating humoral immune responses (Nakae et al., 2001). IL-18 functions in potent stimulation of interferon-γ production enhancing cell toxicity of NK cells, and stimulates T-helper1 cell differentiation (Nakanishi et al., 2001; Biet et al., 2002; Gracie et al., 2003). IL-1ra antagonizes IL-1α

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and IL-1β induced activities by serving as a decoy ligand that binds to the same IL-1R receptor, but without initiating intracellular signal transduction (Dripps et al., 1991; Granowitz et al., 1991).

As the most potent IL-1 cytokine, IL-1β is primarily synthesized not only in monocytes, but also in macrophages, peripheral neutrophilic granulocytes, endothelial cells, fibroblasts, Langerhans cells of the skin, microglia cells, and many other cell types (reviewed by Huising et al., 2004). IL-1β plays key roles in the inflammatory responses, enhancing cell-mediated immunity by inducing the growth and proliferation of lymphocytes, connective tissues, and vascular endothelial cells, and by stimulating immune and inflammatory response effector cells. IL-1 acts synergistically with IL-4 to activate B cells, augmenting the humoral immune response. Along with IL-2 and interferon-γ, it also stimulates non-specific immunity through the activation of NK cells (Kullberg and van der Meer, 1995).

IL-1β is an immunoregulatory cytokine that has the potential to enhance the immune response induced by vaccines (Nash et al., 1993). Recombinant IL-1β is used as an adjuvant for vaccines in sheep (Elhay and Andersen, 1997; Lofthouse et al., 1995), pigs (Blecha et al., 1995), cattle (Reddy et al., 1990), and sea bass (Buonocore et al., 2004, 2005). In spite of its importance and two decades of studies in the mammalian species, interleukin-1 studies in non-mammalian species are a recent event. However, rapid progress has been made in recent years through the use of genomic approaches. IL-1β has been cloned from a number of non-mammalian species recently including chicken (Weining et al., 1998), *Xenopus* (Zou et al., 2000), rainbow trout (Secombes et al., 1998; Zou et al., 1999; Pleguezuelos et al., 2000; Wang et al., 2002, 2004), carp (Fujiki et al., 2000; Engelsma et al., 2001), sea bass (Scapigliati et al., 2001), and small spotted catshark (Bird et al., 2002). Interleukin-1β derived peptides as well as recombinant IL-1β have been shown to induce migration of head kidney leukocytes in vitro, optimal migration of peritoneal leukocytes in vivo and phagocytosis, and enhance resistance against viral haemorrhagic septicemia and bacterial disease caused by *Aeromonas salmonicida* (Peddie et al., 2001, 2003; Hong et al., 2003). Significant increase in survival rates was achieved by intraperitoneal administration of either rIL-1β or IL-1β-derived peptides. Clearly, studies of IL-1β in teleosts not only provide knowledge on gene evolution of immune systems, but also have practical implications. Channel catfish is the primary species of aquaculture in the United States. It is also a classical model for the study of comparative immunology. The catfish immune system is well characterized among fish species, and it is the only fish species where clonal functionally distinct lymphocyte lines have been established (Clem et al., 1990, 1996; Stuge et al., 2000; Miller et al., 1998; Shen et al., 2002). Its IL-1 gene(s) have not been identified, however. In this work, we report molecular cloning of two channel catfish IL-1β cDNAs, sequencing and characterization of their corresponding genes, assignation of IL-1β genes to BACs, and characterization of their expression in various tissues and induced expression after infection of catfish with *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC).

2. Materials and methods

2.1. Identification of putative catfish interleukin-1β cDNA clones

The catfish interleukin-1β cDNA clones were identified through the analysis of expressed sequence tags (EST, Kocabas et al., 2002; Liu, unpublished). BLASTX searches were conducted and homologous sequences to interleukin-1β were recorded with a *p*-value of 10^{-4}. Upon initial identification of interleukin-1β like sequences, the relevant clones were subjected to sequencing analysis.

2.2. Assigning interleukin gene to BAC clones

The interleukin-1β cDNA sequences were used to design overgo probes (Bao et al., 2005, in press; Xu et al., 2005) for hybridization to BAC filters. High-density filters of channel catfish BAC library were purchased from Children's Hospital of the Oakland Research Institute (CHORI, Oakland, CA). Each set of filters contained 10-genome coverage of the channel catfish BAC clones from BAC library CHORI 212 (http://bacpac.chori.org/library.php?id=103). Sequences of the overgo primers are shown in Table 1 and their positions within interleukin cDNA are shown in Fig. 1. Overgo hybridization method was adapted from a web protocol (http://www.tree.caltech.edu/) with modifications (Baoprasertkul et al., 2005). Briefly, overgo primers were selected following a BLAST search against GenBank to screen out repeated sequences and then purchased from Sigma Genosys (Woodlands, Texas). Two hundred nanograms (200 ng) of overgo primers each labeled with 40 μl of a freshly prepared master mix composed of 14.0 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.02 mM dGTP, 0.02 mM dTTP, 20 μCi [α-^{33}P]dCTP, 20 μCi [α-^{33}P]dATP (3000 Ci/mmol, Amersham, Piscataway, NJ), and 5 units of Klenow enzyme (Invitrogen). Labeling reactions were carried out at room temperature for 2 h. After removal of unincorporated nucleotides using a Sephadex G50 spin column, probes were denatured at 95 °C for 10 min and added to the hybridization tubes containing high-density BAC filters. Hybridization was performed at 54 °C for 18 h in hybridization solution (50 ml of 1% BSA, 1 MM EDTA at pH 8.0, 7% SDS, 0.5 mM sodium phosphate, pH 7.2). BAC filters were washed with 2× SSC at room temperature for 15 min, and exposed to X-ray film at −80 °C for 2 days. Positive clones were identified according to the clone distribution instructions from CHORI, and picked out from the channel catfish BAC library.

2.3. Preparation of BAC DNA and Southern blot analysis

After assigning of interleukin-1β to BACs, the putative interleukin-1β positive BAC DNA was isolated with the Perfectprep® BAC 96 BAC DNA isolation kit (Brinkmann Instruments, Inc., Westbury, NY). The isolated BAC DNA was subjected to Southern blot analysis to determine genomic copy numbers based on restriction fingerprinting (Bao et al., 2005, in press; Xu et al., 2005). Briefly, BAC DNA was first digested

Table 1
Primers and their sequences used in this study

Primer name	Sequences (5′ to 3′)	Utilization
Overgo upper primer	AATATTCAGTCCACGGAGTTCACC	Overgo probes
Overgo lower primer	TGAAAAGCTCCTGGTCGGTGAACT	Overgo probes
Gene 1 RT-PCR upper primer	CGGCAGATGTGACCTGCACA	Gene-specific RT-PCR
Gene 1 RT-PCR lower primer	CAGAGTAAAAGCCAGCAGAAG	Gene-specific RT-PCR
Gene 2 RT-PCR upper primer	TGGCAGATGTGACCTGCACG	Gene-specific RT-PCR
Gene 2 RT-PCR lower primer	CAGAGTAAAGGCCAGCAGAAT	Gene-specific RT-PCR
β-Actin RT-PCR upper primer	AGAGAGAAATTGTCCGTGACATC	Internal control for RT-PCR
β-Actin RT-PCR upper primer	CTCCGATCCAGACAGAGTATTTG	Internal control for RT-PCR
BAC sequence primer 1	GGATTAGAAACTGCGTTCTG	BAC sequencing
BAC sequence primer 2	TGACAGTGACTGTGGATTTG	BAC sequencing
BAC sequence primer 3	TCAGAGCAGTCCAGCTTGTC	BAC sequencing
BAC sequence primer 4	TGTAATCGCTTTGCAGAGGC	BAC sequencing
BAC sequence primer 5	CCTGCAAGACAAGGTTGTGC	BAC sequencing
BAC sequence primer 6	GAGGAAGCTTTTCCTTCTGC	BAC sequencing
BAC sequence primer 7	CTTCAGAAACGGCACTGGTG	BAC sequencing
BAC sequence primer 8	TGCACTGGCTTATCGTCATC	BAC sequencing
BAC sequence primer 9	TGGGATATGTGACAGCGCTG	BAC sequencing
BAC sequence primer 10	ATCACCACCTCAAAGGATGACG	BAC sequencing

For positions of the important primers, see Fig. 1.

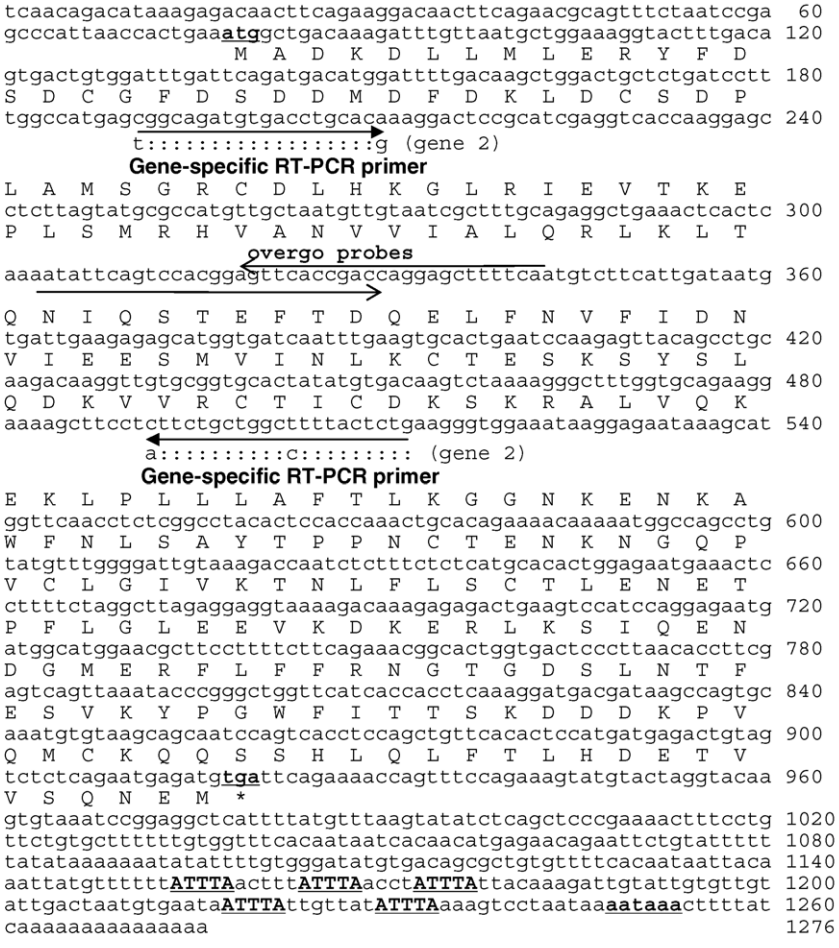

Fig. 1. The nucleotide and deduced amino acid sequences of channel catfish IL-1β cDNA. 5′- and 3′-UTR are shown as small letters. The translation start codon ATG, the translation termination codon TGA, the poly $(A)^+$ signal sequence AATAAA are both bold and underlined. The termination codon is labeled by asterisk. The instability motifs ATTTA are in capital letters, bold, and underlined. The locations of the primers are indicated by underline arrows. While the nucleotide sequence of the second type of IL-1β cDNA is not shown, its sequence at the primer sites is indicated underneath the IL-1β type 1 cDNA sequences.

with restriction endonucleases *Eco*RI and *Hin*dIII, separately, and electrophoresed on a 1% agarose gel. The DNA was transferred to a piece of Immobilon nylon membrane (Millipore, Bedford, MA) by capillary transfer with 0.4 M NaOH overnight. DNA was fixed to the membrane by UV cross-linking using a UV Stratalinker 2400 (Stratagene, La Jolla, CA) with the auto cross-link function. The membrane was washed in 0.5% (w/v) SDS at 65 °C for 15 min and then pre-hybridized in 50% formamide, 5× SSC (Sambrook et al., 1989), 0.1% (w/v) SDS, 5× Denhardt's and 100 μg/ml sonicated and denatured Atlantic salmon sperm DNA (100 μg/ml) overnight. Hybridization was conducted overnight at 42 °C in the same solution with probes added. The interleukin cDNA (accession number DQ157741) was used as the probe. The probe was prepared using the random primer labeling method (Sambrook et al., 1989) with a labeling kit from Roche Diagnostics (Indianapolis, Indiana). The nylon membranes were washed first in 500 ml of 2× SSC for 10 min, followed by three washes in 0.2× SSC with SDS at 0.2% (w/v) at 65 °C for 15 min each. The membranes were then wrapped in Saran wrap and exposed to Kodak BioMax MS film for autoradiography.

2.4. DNA sequencing and sequence analysis

Plasmid DNA containing the IL-1βcDNA was prepared using the alkaline lysis method (Sambrook et al., 1989) using the Qiagen Spin Column Mini-plasmid kits (Qiagen, Valencia, CA). Three microliters of plasmid DNA (about 0.5–1.0 μg) were used in sequencing reactions. Chain termination sequencing was performed using the BigDye sequencing kits (ABI, Foster City, CA). The PCR profiles were: 95 °C for 30 s, 54 °C for 15 s, 60 °C for 4 min for 60 cycles. An initial 5 min denaturation at 95 °C and a 5 min extension at 60 °C were used. For BAC direct sequencing, BAC DNA isolated as described above was used as templates of sequencing using the same sequencing reaction profiles except that 100 cycles were run for cycle sequencing. All sequences were analyzed on an ABI Prism 3130XL automatic sequencer.

Bioinformatic analysis of sequences was conducted by using BLAST and the DNASTAR software package (Serapion et al., 2004). BLAST searches were conducted to determine gene identities, and to determine if the cDNA contained a full open reading frame. DNASTAR software package was used for sequence analysis. MegAlign program of the DNASTAR package was used for sequence alignment using CLUSTAL W. Cluster analysis was conducted using Vector NTI suite of software packages (He et al., 2004; Peatman et al., 2005).

2.5. Phylogenetic analysis

Phylogenetic trees were drawn from ClustalW generated multiple sequence alignments of amino acid sequences using the neighbor-joining method (Saitou and Nei, 1987) within the Molecular Evolutionary Genetics Analysis (MEGA (3.0)) package (Kumar et al., 2004). Data were analyzed using Poisson correction and gaps were removed by complete deletion. The topological stability of the neighbor-joining trees was evaluated by 10,000 bootstrapping replications.

2.6. Fish rearing and bacterial challenge

Channel catfish larvae were reared at the hatchery of the Auburn University Fish Genetics Research Unit. Challenge experiments were conducted as previously described (Dunham et al., 1993) with modifications (Baoprasertkul et al., 2004, 2005; Chen et al., 2005). Briefly, catfish were challenged in a rectangular tank by immersion exposure for 2 h with freshly prepared culture of ESC bacteria, *Edwardsiella ictaluri*. One single colony of *E. ictaluri* was isolated from a natural outbreak in Alabama (outbreak number ALG-02-414) and inoculated into brain heart infusion (BHI) medium and incubated in a shaker incubator at 28 °C overnight. The bacterial concentration was determined using colony forming unit (CFU) per milliliters by plating 10 μl of 10-fold serial dilutions onto BHI agar plates. At the time of challenge, the bacterial culture was added to the tank to a concentration of 3×10^7 CFU/ml. During challenge, an oxygen tank was used to ensure a dissolved oxygen concentration above 5 mg/ml. After 2 h of immersion exposure, 15 fish were randomly taken and placed into a rectangular trough containing pond water with constant water flow through. Replicates of troughs were used to provide one trough for each sampling time point. For the control fish, 15 fish were incubated in a separate rectangular tank with the same fish density as the challenge tanks. The only difference was that ESC bacteria were not added. After 2 h, these control fish were incubated in a separate trough at the same density as the challenged fish.

2.7. Tissue sampling and RNA extraction

Ten tissues were collected from healthy channel catfish including brain, head kidney, intestine, liver, muscle, ovary, skin, spleen, stomach, and trunk kidney. Head kidney and spleen tissues were also collected from challenged fish. Samples were collected from 10 fish at each time point including control (before challenge), and 1, 3, and 7 days after challenge. Samples of each tissue from 10 fish were pooled. The experimental fish were euthanized with tricaine methanesulfonate (MS 222) at 100 mg/l before tissues were collected. Tissues were quick frozen in liquid nitrogen and kept in a −80 °C ultra-low freezer until preparation of RNA. In order to obtain samples representing the average of the 10 fish, the pooled tissue samples were ground with a mortar/pestle to fine powder and thoroughly mixed. A fraction of the tissue samples was used for RNA isolation. RNA was isolated following the guanidium thiocyanate method (Chomczynski and Sacchi, 1987) using the Trizol reagents kit from Invitrogen following manufacturer's instructions. Extracted RNA was stored in a −70 °C freezer until used as template for reverse transcriptase-PCR (RT-PCR).

2.8. Reverse transcriptase-PCR

Total RNA was used for reverse transcriptase-PCR reactions. The RT-PCR reaction was conducted using two-step RT-PCR.

RT was conducted by using M-MuLV reverse transcriptase (New England Biolabs). RT reactions were conducted in 40 μl containing 4 μg DNase I-treated RNA, 4 μl (40 uM) oligo dT primers, 8 μl (2.5 mM each) dNTPs, 1 μl RNase inhibitor, 1× RT reaction buffer, and 200 units of RT. Detailed procedures followed the instructions of the manufacturer. After RT reaction, 1 μl of the RT products was used as templates for PCR using Jump-Start Taq polymerase (Sigma, St. Louis, MO). The reactions also included the primers of β-actin (Table 1), serving as an internal control. The reactions were completed in a thermocycler with the following thermo-profiles: denaturation at 94 °C for 3 min followed by 32 cycles for the analysis of tissue distribution of interleukin-1β expression, and 28 cycles for the analysis of bacterial induced interleukin expression with 94 °C for 30 s, 56 °C for 15 s, 72 °C for 40 s. Upon the completion of PCR, the reaction was incubated at 72 °C for an additional 5 min. The RT-PCR products were analyzed by electrophoresis on a 1.0% agarose gel and documented with a Gel Documentation System (Nucleotech Corp., CA).

3. Results

3.1. cDNA cloning of channel catfish interleukin-1β

Channel catfish sequences homologous to interleukin-1β were initially identified from expressed sequence tags. Through BLASTX searches, four clones, IpHdk_45_n08, IpHdk_46_b09, IpInt_56_k12, and IpSpn_66_c04, were initially identified as homologous to interleukin-1β with significant sequence similarities ($<10^{-8}$) to the query sequence. Additional searches of the dbEST database identified two additional clones, IpSpn01597 (accession number BM425330) and IpSpn00477 (accession number BM424711), from previously sequenced catfish ESTs (Kocabas et al., 2002) as interleukin-1β. Cluster analysis indicated that the cDNAs belong to two groups, perhaps suggesting the presence of highly different alleles, or the presence of two interleukin-1β genes in channel catfish. The cDNA clones were completely sequenced, and the complete cDNA sequences have been deposited to GenBank with accession numbers DQ157741 and DQ157742.

As shown in Fig. 1 and summarized in Table 2, both cDNAs of channel catfish interleukin-1β encode 280 amino acids. Their 3′-untranslated region was slightly different. cDNAs from gene 1 and gene 2 both harbor the typical AAUAAA polyadenylation signal, 13 and 18 bp, respectively, upstream from the poly A tail. There are five copies of ATTTA instability motif at the 3′-UTR of the cDNA encoded by gene 1, but seven copies existed in the cDNA encoded by gene 2, both highly characteristic of cytokine transcripts (Fig. 1).

The channel catfish IL-1β transcripts encoded by the two genes are highly similar with 94.3% amino acid sequence identity. Alignments of IL-1β genes from various species indicated a more rapid rate of evolution of fish IL-1β genes (Table 3). Among mammals, IL-1β genes exhibited a similarity of at least 59%, whereas the channel catfish IL-β genes shared only approximately 31% sequence identities with the salmonids IL-β genes. Sequence alignments of selected fish IL-β genes revealed major sequence divergence between the taxa. IL-1β genes share high sequence identities among the “carps”, the salmonids, and the catfish, but inter-taxa similarity is low (Fig. 2). An interesting observation is the greater similarity between the mammalian IL-1β genes and the *Xenopus laevis* IL-1β than between fish IL-1β and the *Xenopus* IL-1β (Table 3).

Table 2
Comparison of the cDNAs encoded by the two channel catfish IL-1β genes

	cDNA of gene 1	cDNA of gene 2
Accession number	DQ157741	DQ157742
cDNA length (bp)	1261	1231
ORF encoding amino acids	280 amino acids	280 amino acids
5′-untranslated region (bp)	≥77	≥61
3′-untranslated region (bp)	341	327
Presence of the first untranslated exon	Yes	Yes
Position of AAUAAA poly A signal	13 bp upstream of poly A tail	18 bp upstream of poly A tail
AUUUA instability motifs	5 copies at 3′-UTR	7 copies at 3′-UTR

Phylogenetic analysis suggested that all the fish IL-1β sequences fell into a single clade whereas the mammalian sequences fell into another. Yet, the chicken and *Xenopus* IL-1β did not fall into either clade though they were closer to the mammalian sequences (Fig. 3). The phylogenetic analysis indicated that the catfish IL-1β genes may have been from a recent gene duplication event as they are more related to each other than to any other IL-1β genes. Similarly, there may be recent gene duplications of the IL-1β locus in the carp and swine genomes as well (Fig. 3).

3.2. Assigning interleukin-1β to BACs

Bacterial artificial chromosome libraries serve as the basis for BAC-based physical maps. In channel catfish, a physical mapping project has been initiated. Therefore, mapping genes to linkage maps and assigning the same genes to BACs is of interest for the alignment of physical and linkage maps. In addition, assigning genes to BACs would allow comparative genome analysis in the genome neighborhood. As part of comparative genomics efforts in channel catfish, we are interested in assigning known genes to BACs. Overgo probes homologous to interleukin-1β were used to assign interleukin-1β gene(s) to BAC clones. Hybridization of interleukin-1β overgo probes to high-density 10× genome coverage BAC filters resulted in identification of 13 positive BAC clones, of which 11 BACs could be grown for the isolation of BAC DNA. Subsequent Southern blot analysis confirmed that all 11 BACs contained interleukin-1β genes (Table 4).

3.3. Interleukin-1β gene is duplicated in the catfish genome

In order to determine the gene copy numbers, BAC DNA from the interleukin-1β positive clones were isolated and subjected

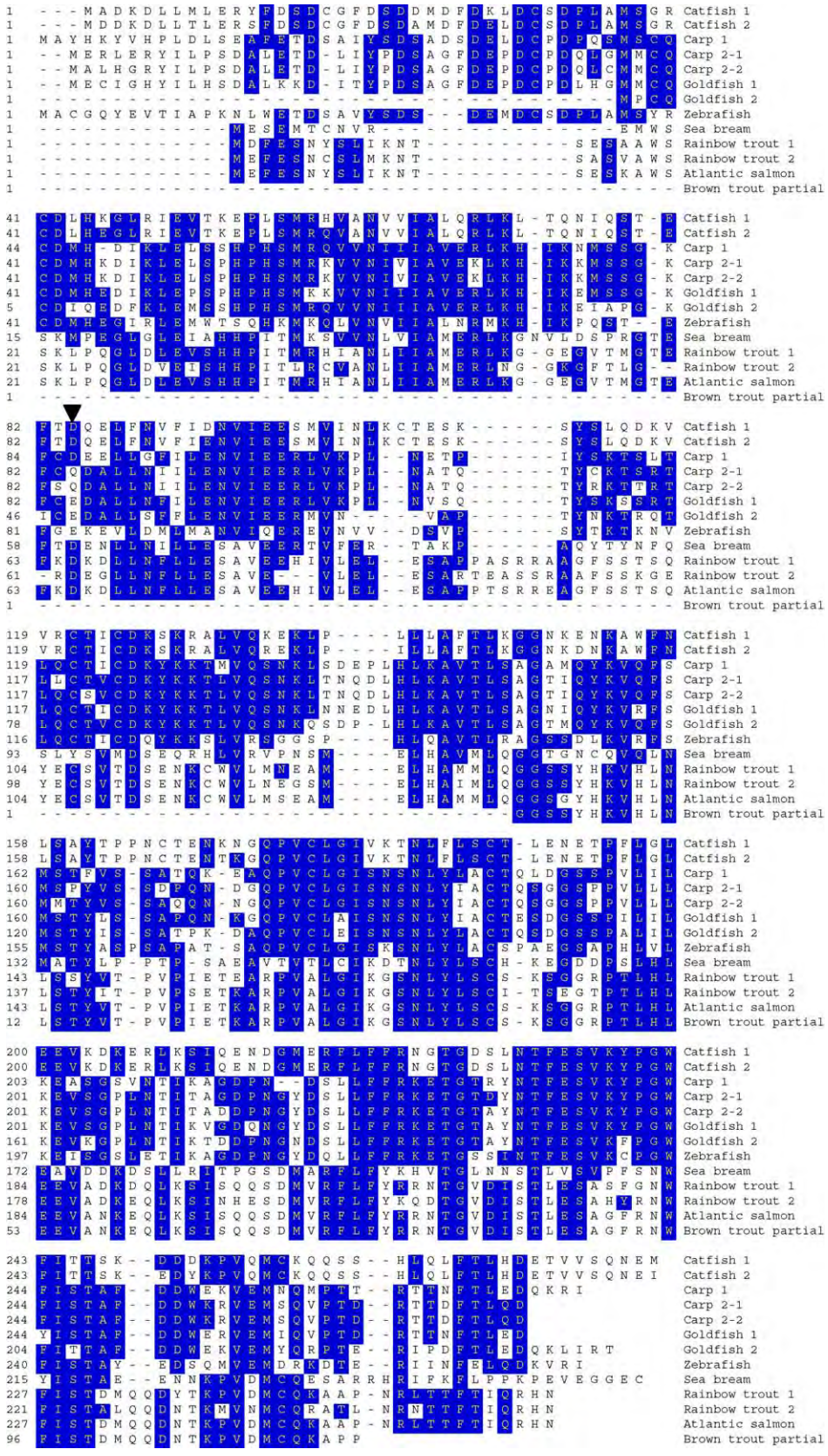

Fig. 2. Sequence alignments of selected fish IL-1β sequences. The amino acid sequences were extracted from GenBank and aligned using ClustalW using DNAStar program. The accession numbers for the sequences are: carp IL-1β(Carp 1), CAB52366; carp IL-1β 2-1 (Carp 2-1), CAC19887; carp IL-1β 2-2 (Carp 2-2), CAC19888; zebrafish IL-1β, AAH98597; goldfish IL-1β-1, CAD12102; goldfish IL-1β-2, CAD12103; rainbow trout IL-1β 1, CAC83518; rainbow trout IL-1β 2, CAB53541; Atlantic salmon IL-1β, AAT36642; brown trout IL-1β, AAX45349; gilthead seabream IL-1β, CAD11603. The solid triangle indicates the suggested IL-1β cleavage site within gilthead seabream (Pelegrin et al., 2004).

Table 3
Pairwise similarities of selected IL-1β genes

	Ip2	Cc1	Cc2	Ca1	Ca2	Om1	Om2	Ss	Xl	Gg	Bt	Oa	Sus	Hs
Ip1	94.3	38.2	37.9	36.4	37.8	31.5	31.5	31.9	19.3	19.5	22.2	21.1	22.1	22.7
Ip2	***	38.2	37.5	37.5	38.2	31.9	32.3	32.3	19.6	20.6	20.7	21.4	21.7	21.9
Cc1		***	93.4	82.4	71.4	26.9	26.0	26.5	20.6	17.2	20.3	20.7	23.6	21.2
Cc2			***	80.9	71.8	26.9	25.2	26.9	21.0	17.6	20.3	20.7	23.2	18.6
Ca1				***	74.8	26.2	26.8	26.5	25.4	19.5	19.2	18.8	22.8	19.3
Ca2					***	29.0	29.0	29.4	24.8	14.7	23.1	20.6	23.9	23.9
Om1						***	76.4	95.0	23.8	26.2	20.4	19.6	22.3	24.2
Om2							***	77.2	22.4	26.8	20.1	20.9	20.1	16.1
Ss								***	23.5	26.2	20.4	20.4	22.7	25.0
Xl									***	34.1	34.2	34.6	28.8	32.3
Gg										***	26.7	27.1	24.7	25.5
Bt											***	93.2	70.3	60.2
Oa												***	70.3	59.0
Sus													***	59.9

Note that high sequence identities exist among mammals, among fish within the *Cyprinidae*, *Salmonidae*, and *Ictaluridae*, but low sequence identities exist among the groups. Species are indicated by letter abbreviations as the following: *Ip*, *Ictalurus punctatus*; *Cc*, *Cyprinus carpio*; *Ca*, *Carassius auratus*; *Om*, *Oncorhynchus mykiss*; *Ss*, *Salmo salar*; *Xl*, *Xenopus laevis*; *Gg*, *Gallus gallus*; *Bt*, *Bos Taurus*; *Oa*, *Ovis aries*; *Sus*, *Sus scrofa*; *Hs*, *Homo sapiens*.

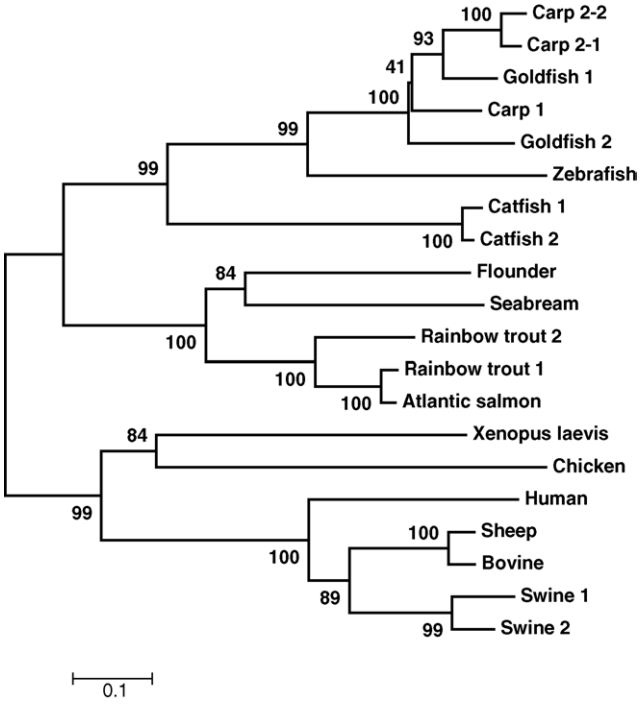

Fig. 3. Phylogenetic tree constructed from alignments of selected IL-1β sequences with the following accession numbers: carp IL-1β, CAB52366; carp IL-1β 2-1, CAC19887; carp IL-1β 2-2, CAC19888; zebrafish IL-1β, AAH98597; goldfish IL-1β, CAD12102; goldfish IL-1β, CAD12103; rainbow trout IL-1β, CAC83518; rainbow trout IL-1β, CAB53541; Atlantic salmon IL-1β, AAT36642; gilthead seabream IL-1β, CAD11603; human, AAA36106; *Xenopus laevis* IL-1β, AAH92346; sheep IL-1β, CAA40293; swine IL-1β 1, NP_999220; swine IL-1β 2, NP_001005149; bovine IL-1β, CAA31018; chicken IL-1β, NP_989855. Numbers on the branches are bootstrapping values as obtained by 10,000 bootstrap replications.

to Southern blot analysis after digestion with two restriction enzymes, *Eco*RI (not shown), and *Hin*dIII. As we previously described, determination of gene copy numbers using BAC-based Southern blot depends on the number of restriction patterns, not the number of bands (Xu et al., 2005; Bao et al., 2005, in press; Baoprasertkul et al., 2005). Southern blot analysis of the interleukin-1β BAC clones indicated the presence of two patterns of restriction fingerprints using either *Hin*dIII (Fig. 4) or *Eco*R1 (not shown). However, examination of the Southern blot hybridization patterns reveal that 2 of the 11 clones harbor a partial band pattern shared with the remaining nine clones, characteristic of partial overlapping genomic regions in different BAC clones. In other words, the two clones may have included only a part of the genomic segments homologous to the probes. Considering the relatively small size of the interleukin-1β gene as compared to the average size of the BAC insert of the channel catfish BAC library (161 kbp), it is likely that two tandem copies of interleukin genes exist in the catfish genome. This speculation was supported by the hybridization patterns using *Eco*R1. In this case, nine clones produced two Southern blot bands with almost equal sizes while the other two clones (corresponding to the two clones with partial hybridization pattern using *Hin*dIII) produced one band sharing the same size with the larger band of the other nine clones. This suggested that the two copies of the interleukin-1β genes could be located within the *Eco*R1 bands, with one copy represented by each of the two bands.

Genomic sequencing confirmed the presence of two copies of interleukin-1β genes in the catfish genome. Subclones of the BAC clone, BAC51_a13, using *Eco*R1 restriction endonuclease were directly sequenced. Two distinct genomic sequences were generated. The complete genomic sequences have been

Table 4
IL-1β positive BAC clones as obtained by overgo hybridization to high-density BAC filters

IL-1 beta positive BAC clones	17_j01, 28_a04, 39_p01, 39_p21, 42_p18, 51_a13, 72_a17, 89_L17, 102_f24, 175_L22, 187_011, 93_c01, 94_o05
IL-1 beta gene 1 positive BAC clones	17_j01, 28_a04, 39_p01, 39_p21, 51_a13, 72_a17, 89_L17, 102_f24, 175_L22
IL-1 beta gene 2 positive BAC clones	17_j01, 28_a04, 39_p01, 39_p21, 42_p18, 51_a13, 72_a17, 89_L17, 102_f24, 175_L22, 187_011

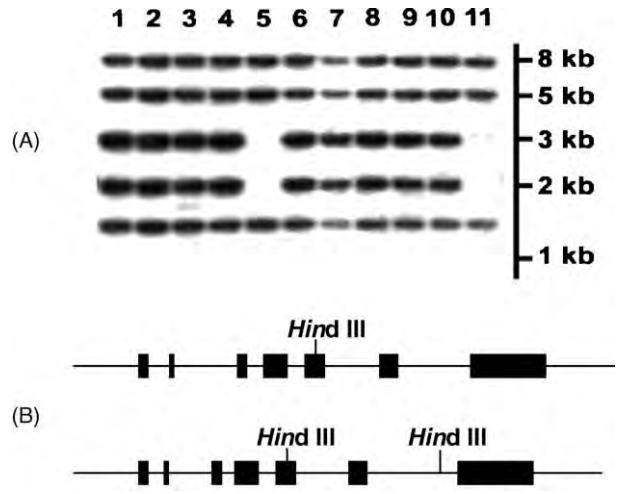

Fig. 4. Demonstration of the presence of duplicated IL-1β genes in channel catfish. (A) Southern blot analysis using BAC clones. BAC DNAs were digested with *Hin*dIII, electrophoresed through a 0.8% agarose gel, transferred to nylon membrane, and hybridized to IL-1β cDNA probes. Lanes 1–11 contained BAC DNA from 11 different BAC clones. Molecular weight is indicated on the right margin. (B) Illustration of the channel catfish genomic structure and the restriction sites of *Hin*dIII as revealed by sequence analysis of a single BAC clone. Solid rectangles represent exons, lines indicate introns. The positions of *Hin*dIII are indicated above the gene structure.

deposited to GenBank with accession numbers of DQ160229 and DQ160230, respectively. Sequence analysis of the genomic sequences revealed the presence of one *Hin*dIII site on exon 5 of interleukin-1β gene 1, whereas two *Hin*dIII sites existed for interleukin-1β gene 2, one was at the same location on exon 5, and the other was gained in intron 6. Such restriction sites would result in the generation of two bands from gene 1 and three bands from gene 2 when complete cDNA was used as a probe (Fig. 4B). Taken together, the Southern blot analysis data and the sequence data confirmed the presence of two tandem genomic copies of interleukin-1β in the catfish genome.

3.4. Genomic organization and sequence variation of the interleukin-1β genes

Both channel catfish interleukin-1β genes have seven exons and six introns, a feature that appears to be conserved of the IL-1β gene organization, with the exception of a rainbow trout IL-1β gene that has six exons (Zou et al., 1999). The two catfish IL-1β genes are highly conserved in gene structure and organization. As shown in Table 5, all the exons shared the same sizes except the last exon that included more divergent sequences at the 3′-UTR. The first exon could have shared the same sizes, but the transcription initiation site was not determined. Using existing sequence information, the first exon of both genes could be longer by approximately some 60 bp because alignment of the two genomic sequences revealed extension of almost identical sequences for another 64 bp beyond the cDNA sequences of gene 1.

The two catfish genes also share very high sequence identities. The exon sequences shared extremely high sequence identities with exception of single nucleotide polymorphism sites

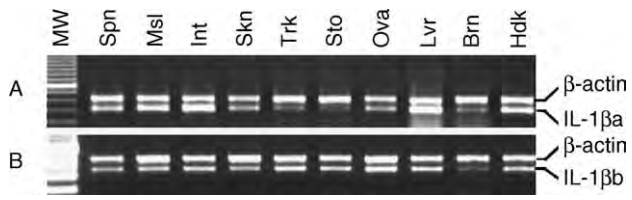

Fig. 5. RT-PCR analysis of IL-1β gene expression in various tissues using gene-specific RT-PCR primers (for details, see Fig. 1) for gene 1 (A) and gene 2 (B). RT-PCR products were analyzed on an agarose gel. Tissues were specified on the top of the gel, with molecular weight (MW), spleen (Spln), muscle (Msl), intestine (Int), skin (Skn), trunk kidney (Trk), stomach (Sto), ovary (Ova), liver (Lvr), brain (Brn), and head kidney (Hdk). The positions of the RT-PCR amplified bands of beta-actin and IL-1β are indicated on the right margin.

(Table 5). Not only are the exon sequences conserved, but also the intronic sequences. Sequence alignment of the two channel catfish IL-β genes indicated a high level of sequence similarities of the introns. What is most striking was the fact that all introns appeared to be highly conserved on both ends, but divergent in the middle. In most cases, stretches of 40–80 bp of almost identical sequences exist on both sides of the introns, intervening the much more divergent portions of the middle part of the introns. In spite of the very high level of sequence identities in both the exons and introns, there is little evidence of sequence conservation in the 5′-upstream sequences containing the promoter, nor is any evidence of sequence conservation after the poly A signals.

3.5. Expression of interleukin-1β genes in normal catfish tissues

RT-PCR analysis using total RNA from various normal tissues of channel catfish indicated that the two catfish IL-1β genes exhibit different profiles of tissue expression. Gene 1 was expressed highly in the liver, head kidney, spleen, intestine, and muscle, but its expression was lower in the stomach, brain, ovary, skin, and trunk kidney. Gene 2 was expressed uniformly in all tested tissues except that it was expressed at lower levels in the brain (Fig. 5).

3.6. Expression of interleukin genes in bacterial infected tissues

As a pro-inflammatory cytokine, interleukin-1β should be involved in inflammatory responses after infection. To determine its specific expression after infection with a bacterial pathogen of catfish, *Edwardsiella ictaluri*, RT-PCR was conducted using total RNA isolated from head kidney and spleen at different times after infection. As shown in Fig. 6, *E. ictaluri* infection elicited sharp increases in transcripts of interleukin-1β both in the head kidney and in the spleen. However, the two genes exhibited quite different profiles of bacterial induced up-regulation. Gene 1 was drastically up-regulated in both the head kidney and the spleen, but gene expression induction was more rapid in the spleen. A sharp increase in interleukin-1β RNA was observed 1 day after infection in the spleen, but drastic induction of interleukin-1β was not observed in the head kidney until 3 days after infection. In contrast, induction of gene 2

Table 5
Comparison of the channel catfish IL-1β gene 1 (accession number DQ160229) and gene 2 (accession number DQ160230)

Exon/Intron	Exon size (gene 1)	Exon size (gene 2)	Intron size (gene 1)	Intron size (gene 2)	Exon similarity
1[a]	≥75	≥59	175	188	4 SNPs
2	34	34	491	343	2 SNPs
3	79	79	103	87	4 SNPs
4	181	181	111	110	4 SNPs
5	168	168	536	417	5 SNPs
6	140	140	579	713	5 SNPs
7	584	570			5 SNPs[b]

[a] The transcriptional start sites were not determined; the numbers reflect existing evidence from cDNA sequence information.
[b] Number reflects only coding region of the last exon.

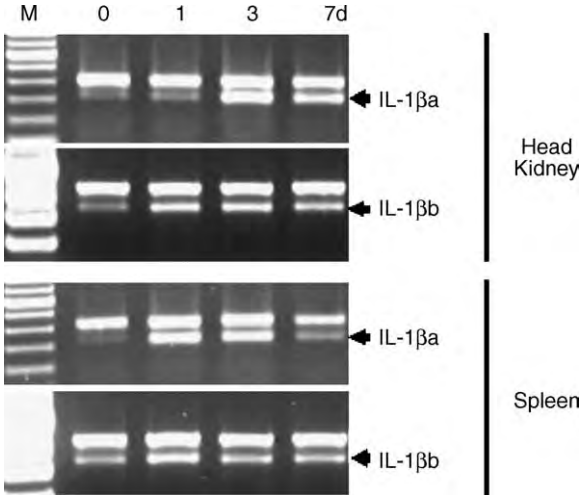

Fig. 6. RT-PCR analysis of IL-1β gene expression after bacterial infection with Edwardsiella ictaluri in head kidney (upper panel) and spleen (lower panel). Tissues of head kidney and spleen were collected at 0 h (uninfected control), 1, 3, and 7 days after infection as indicated at the top of the gel. RT-PCR was conducted using gene-specific RT-PCR primers (for details, see Fig. 1) for gene 1 (IL-1βa) and gene 2 (IL-1βb), as specified on the figure. The positions of the RT-PCR amplified bands of IL-1β are indicated on the right margin. Two bands from each RT-PCR are clearly visible; one is IL-1β product, and the other is beta-actin product that is not labeled.

expression appeared rapidly in both the head kidney and spleen, with increased interleukin-1β expression 1 day after infection. However, the extent of induction was much less dramatic for gene 2 than for gene 1 (Fig. 6). Clearly, the two copies of the channel catfish interleukin-1β genes not only exhibit differences in their spatial pattern of expression, but also in patterns of expression after bacterial infection.

4. Discussion

In the present study, we characterized the two cDNAs and their corresponding encoding genes in the channel catfish genome. The channel catfish IL-1β genes were found to be duplicated in the catfish genome in a tandem fashion. Both genes were widely expressed, but exhibit different expression profiles. The IL-1β genes were mapped to BAC clones. As the catfish physical mapping project has been initiated, this information will soon be useful for integration of the physical map with linkage maps.

IL-1β gene duplication in channel catfish is an unexpected finding. In spite of previous reports of the presence of two IL-1β genes in the carp, goldfish, and rainbow trout, those fish has been generally regarded as tetraploid fish, whereas channel catfish is a diploid fish. Evolutionarily, channel catfish is closely related to zebrafish. BLAST search against the draft zebrafish genome sequences indicated the presence of only one IL-1β like sequence in the zebrafish genome located in Contig: NW_653018.1, which is not yet assigned to a chromosome. A search of the chicken genome draft sequence (Build 1.1) suggests that chicken also has a single copy of interleukin-1β gene. This may suggest that the channel catfish IL-1β genes were derived from a recent gene duplication. This is supported by its tandem presence with very high sequence identity and identical sizes of all but the last exon of the two genes. Such species-specific gene duplication was previously observed in mammals. Human has only one copy of IL-1β gene located on its chromosome 2, but two IL-1β genes exist in the swine genome (Vandenbroeck et al., 1994).

The two channel catfish IL-1β genes are arranged in tandem. Through BAC-based Southern blot analysis, we have gained strong evidence that the two IL-1β genes in channel catfish are present in the same BACs. The Southern blot data, when taken together with the sequence information, demonstrate that the two copies of the channel catfish IL-1β genes are closely linked in tandem, although the exact distance between the two catfish genes was not estimated. Based on the frequency with which only one copy of the gene was included in the BAC (2/11), the two genes should be fairly close with a statistical estimated distance of approximately 30 kbp apart, assuming restriction digestion was totally random during construction of the BAC library. This arrangement is quite peculiar as to its evolutionary origin. Despite the report of two genes in rainbow trout, carp, and goldfish, their genomic arrangements are not known. It would be interesting to compare the genomic arrangements of the duplicated genes, thereby providing information as to if the duplicated genes in several other fish species are related to the tetraploidy of their genomes. In the case of channel catfish, identification and sequencing of the two IL-1β genes from a single BAC clone confirmed the presence of the two copies of the IL-1β genes on a single chromosome, excluding the possibility of them being allelic variations.

We previously reported the methods of using BAC-based Southern as a way of gene copy number determination. In

previous papers (Bao et al., 2005, in press; Xu et al., 2005; Baoprasertkul et al., 2005), we discussed the relationship of band patterns or fingerprint pattern as the most accurate way for the determination of gene copy numbers. If two genes are from different genome contexts, obviously the restriction fingerprints will be different. Thus, the number of restriction patterns, but not the number of bands, defines the gene copy numbers. This approach is superior to the traditional Southern blot-based approach for determination of gene copy numbers. In traditional methods using Southern blot analysis, researchers determine gene copy numbers based on how many restriction bands one would obtain using several restriction endonucleases, most often 6-bp cutters. The assumption is that at least some 6-bp cutters would not cut within the gene and, therefore, a single band should result. This is certainly true because a 6-bp cutter restriction enzyme should cleave DNA at a frequency of $1/4^6$ bp (once every 4096 bp) that is larger than the sizes of many genes. However, because most researchers use no more than a few enzymes, this traditional approach can certainly result in mistaken determination of copy numbers if the few restriction endonucleases all cut within the gene.

Our recent analysis using BAC-based Southern blot demonstrated also a greater capacity for determination of gene copy numbers under the scenario of tandemly duplicated genes. When two genes are duplicated nearby in tandem, determination of gene copy number can be problematic based on Southern blot analysis using traditional approaches. Obviously, hybridization signal intensity could be twice as strong as for a single copy, but determination based on band intensities is of questionable accuracy. If genes are duplicated in tandem nearby such that they are included in the same BACs, the chances of detecting these duplicated genes using BAC-based Southern blot analysis depend on the presence of BAC clones with only one copy of the genes. This is reflected in the BAC-based Southern blot patterns. In the BACs where a copy of the genes is missing, its BAC-based Southern blot pattern should be a smaller part of the larger pattern seen in BACs containing all copies. One notable difficulty with this approach is when the gene of interest is located at the end of BACs. However, in those cases the likelihood of having two BAC clones with identical ends is extremely small. We have successfully determined copy numbers using this approach to deal with tandemly duplicated genes here for IL-1β genes, as well as for NK-lysin genes (Wang et al., 2006).

Sequence analysis suggested that IL-1β genes experienced rapid evolution within the teleost branch. While from the mammals to *Xenopus* a moderate level of sequence identity (>30%) was observed, this level of sequence conservation was not retained among the teleost IL-1β sequences. Despite very high sequence identity between/among IL-1β genes in closely related fish species, rapid sequence divergence was observed even between the carps and the catfish, which are evolutionarily highly related. This rapid sequence divergence in fish may explain in part the difficulties in the identification of other IL-1 family members in teleosts.

Through the use of gene-specific PCR primers, we demonstrated that both copies of the catfish IL-1β genes were expressed. They were both expressed in a wide range of tissues, but their levels of expression in the tissues were different. Most striking was the induced expression of the IL-1β genes after bacterial infection. Although both IL-1β genes exhibited infection-induced up-regulation, the extent to which they were induced was quite different. IL-1β gene 1 was drastically induced after infection in both the head kidney and spleen, whereas the induction of gene 2 was not so dramatic. Such differences of gene expression could be a direct reflection of differences in their regulatory sequences including the promoter. In spite of high sequence identity between the two channel catfish IL-1β genes, their upstream sequences do not share any significantly similar sequences. Future studies should be warranted to investigate the control regions for the differential regulation of the two genes.

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References

Bao, B., Peatman, E., Li, P., He, C., Liu, Z.J., 2005. Catfish hepcidin gene is expressed in a wide range of tissues and exhibits tissue-specific upregulation after bacterial infection. Dev. Comp. Immunol. 29, 939–950.

Bao, B., Peatman, E., Xu, P., Li, P., Zeng, H., He, C., Liu, Z.J. The catfish liver-expressed antimicrobial peptide 2 (LEAP-2) gene is expressed in a wide range of tissues and developmentally regulated. Mol. Immunol., in press.

Baoprasertkul, P., He, C., Peatman, E., Zhang, S., Li, P., Liu, Z.J., 2005. Constitutive expression of three novel catfish CXC chemokines: homeostatic chemokines in teleost fish. Mol. Immunol. 42, 1355–1366.

Baoprasertkul, P., Peatman, E., Chen, L., He, C., Kucuktas, H., Li, P., Simmons, M., Liu, Z., 2004. Sequence analysis and expression of a CXC chemokine in resistant and susceptible catfish after infection of *Edwardsiella ictaluri*. Dev. Comp. Immunol. 28, 769–780.

Beutler, B., 2004. Innate immunity: an overview. Mol. Immunol. 40, 845–859.

Biet, F., Locht, C., Kremer, L., 2002. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. J. Mol. Med. 80, 147–162.

Bird, S., Zou, J., Wang, T., Munday, B., Cunningham, C., Secombes, C.J., 2002. Evolution of interleukin-1 beta. Cytokine Growth Factor Rev. 13, 483–502.

Blecha, F., Reddy, D.N., Chitko-McKown, C.G., McVey, D.S., Chengappa, M.M., Goodband, R.D., Nelssen, J.L., 1995. Influence of recombinant bovine interleukin-1 beta and interleukin-2 in pigs vaccinated and challenged with *Streptococcus suis*. Vet. Immunol. Immunopathol. 44, 329–346.

Bohana-Kashtan, O., Ziporen, L., Donin, N., Kraus, S., Fishelson, Z., 2004. Cell signals transduced by complement. Mol. Immunol. 41, 583–597.

Bottino, C., Moretta, L., Pende, D., Vitale, M., Moretta, A., 2004. Learning how to discriminate between friends and enemies, a lesson from Natural Killer cells. Mol. Immunol. 41, 569–575.

Buonocore, F., Forlenza, M., Randelli, E., Benedetti, S., Bossu, P., Meloni, S., Secombes, C.J., Mazzini, M., Scapigliati, G., 2005. Biological activity of sea bass (*Dicentrarchus labrax* L.) recombinant interleukin-1 beta. Mar. Biotechnol. (Epub ahead of print).

Buonocore, F., Mazzini, M., Forlenza, M., Randelli, E., Secombes, C.J., Zou, J., Scapigliati, G., 2004. Expression in *Escherchia coli* and purification of

sea bass (*Dicentrarchus labrax*) interleukin 1beta, a possible immunoadjuvant in aquaculture. Mar. Biotechnol. 6, 53–59.

Chen, L., He, C., Baoprasertkul, P., Xu, P., Li, P., Serapion, J., Waldbieser, G., Wolters, W., Liu, Z., 2005. Analysis of a catfish gene resembling interleukin-8: cDNA cloning, gene structure, and expression after infection with *Edwardsiella ictaluri*. Dev. Comp. Immunol. 29, 135–142.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Clem, L.W., Bly, J.E., Wilson, M., Chinchar, V.G., Stuge, T., Barker, K., Luft, C., Rycyzyn, M., Hogan, R.J., van Lopik, T., Miller, N.W., 1996. Fish immunology: the utility of immortalized lymphoid cells—a mini review. Vet. Immunol. Immunopathol. 54, 137–144.

Clem, L.W., Bly, J.E., Ellsaesser, C.F., Lobb, C.J., Miller, N.W., 1990. Channel catfish as an unconventional model for immunological studies. J. Exp. Zool. Suppl. 4, 123–125.

Debets, R., Timans, J.C., Homey, B., Zurawski, S., Sana, T.R., Lo, S., Wagner, J., Edwards, G., Clifford, T., Menon, S., Bazan, J.F., Kastelein, R.A., 2001. Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. J. Immunol. 167, 1440–1446.

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., Eisenberg, S.P., 1991. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J. Biol. Chem. 266, 10331–10336.

Dunham, R.A., Brady, Y., Vinitnantharat, S., 1993. Response to challenge with *Edwardsiella ictaluri* by channel catfish, *Ictalurus punctatus*, selected for resistance to *E. ictaluri*. J. Appl. Aquacult. 3, 211–222.

Elhay, M.J., Andersen, P., 1997. Immunological requirements for a subunit vaccine against tuberculosis. Immunol. Cell. Biol. 75, 595–603.

Engelsma, M.Y., Stet, R.J., Schipper, H., Verburg-van Kemenade, B.M., 2001. Regulation of interleukin 1 beta RNA expression in the common carp *Cyprinus carpio* L. Dev. Comp. Immunol. 25, 195–203.

Fujiki, K., Shin, D.H., Nakao, M., Yano, T., 2000. Molecular cloning and expression analysis of carp (*Cyprinus carpio*) interleukin-1 beta, high affinity immunoglobulin E Fc receptor gamma subunit and serum amyloid A. Fish Shellfish Immunol. 10, 229–242.

Gasque, P., 2004. Complement: a unique innate immune sensor for danger signals. Mol. Immunol. 41, 1089–1098.

Gracie, J.A., Robertson, S.E., McInnes, I.B., 2003. Interleukin-18. J. Leukoc. Biol. 73, 213–224.

Granowitz, E.V., Clark, B.D., Mancilla, J., Dinarello, C.A., 1991. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. J. Biol. Chem. 266, 14147–14150.

He, C., Peatman, E., Baoprasertkul, P., Kucuktas, H., Liu, Z.J., 2004. Multiple CC chemokines in channel catfish and blue catfish as revealed by analysis of expressed sequence tags. Immunogenetics 56, 379–387.

Hong, S., Peddie, S., Campos-Perez, J.J., Zou, J., Secombes, C.J., 2003. The effect of intraperitoneally administered recombinant IL-1 beta on immune parameters and resistance to *Aeromonas salmonicida* in the rainbow trout (*Oncorhynchus mykiss*). Dev. Comp. Immunol. 27, 801–812.

Huising, M.O., Stet, R.J., Savelkoul, H.F., Verburg-van Kemenade, B.M., 2004. The molecular evolution of the interleukin-1 family of cytokines: IL-18 in teleost fish. Dev. Comp. Immunol. 28, 395–413.

Khalturin, K., Panzer, Z., Cooper, M.D., Bosch, T.C., 2004. Recognition strategies in the innate immune system of ancestral chordates. Mol. Immunol. 41, 1077–1087.

Kocabas, A.M., Li, P., Cao, D., Karsi, A., He, C., Patterson, A., Ju, Z., Dunham, R.A., Liu, Z., 2002. Expression profile of the channel catfish spleen: analysis of genes involved in immune functions. Mar. Biotechnol. 4, 526–536.

Kullberg, B.J., van der Meer, J.W.M., 1995. Cytokines in the Treatment of Infectious Diseases: Options for the Modulation of Host Defense. Kluwer, Dordrecht.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.

Lofthouse, S.A., Andrews, A.E., Barcham, G.J., Nash, A.D., 1995. Parameters related to the application of recombinant ovine interleukin-1 beta as an adjuvant. Vaccine 13, 1277–1287.

Miller, N., Wilson, M., Bengten, E., Stuge, T., Warr, G., Clem, W., 1998. Functional and molecular characterization of teleost leukocytes. Immunol. Rev. 166, 187–197.

Nakae, S., Asano, M., Horai, R., Iwakura, Y., 2001. Interleukin-1 beta, but not interleukin-1 alpha, is required for T-cell-dependent antibody production. Immunology 104, 402–409.

Nakanishi, K., Yoshimoto, T., Tsutsui, H., Okamura, H., 2001. Interleukin-18 regulates both Th1 and Th2 responses. Annu. Rev. Immunol. 19, 423–474.

Nash, A.D., Lofthouse, S.A., Barcham, G.J., Jacobs, H.J., Ashman, K., Meeusen, E.N., Brandon, M.R., Andrews, A.E., 1993. Recombinant cytokines as immunological adjuvants. Immunol. Cell. Biol. 71, 367–379.

Peatman, E., Bao, B., Baoprasertkul, P., Liu, Z.J., 2005. In silico identification and expression analysis of 12 novel CC chemokines in catfish. Immunogenetics 57, 409–419.

Peddie, S., McLauchlan, P.E., Ellis, A.E., Secombes, C.J., 2003. Effect of intraperitoneally administered IL-1 beta-derived peptides on resistance to viral haemorrhagic septicaemia in rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Organ. 56, 195–200.

Peddie, S., Zou, J., Cunningham, C., Secombes, C.J., 2001. Rainbow trout (*Oncorhynchus mykiss*) recombinant IL-1 beta and derived peptides induce migration of head–kidney leucocytes in vitro. Fish Shellfish Immunol. 11, 697–709.

Pelegrin, P., Chaves-Pozo, E., Mulero, V., Meseguer, J., 2004. Production and mechanism of secretion of interleukin-1 beta from the marine fish gilthead seabream. Dev. Comp. Immunol. 28, 229–237.

Philpott, D.J., Girardin, S.E., 2004. The role of Toll-like receptors and Nod proteins in bacterial infection. Mol. Immunol. 41, 1099–1108.

Pleguezuelos, O., Zou, J., Cunningham, C., Secombes, C.J., 2000. Cloning, sequencing, and analysis of expression of a second IL-1beta gene in rainbow trout (*Oncorhynchus mykiss*). Immunogenetics 51, 1002–1011.

Reddy, D.N., Reddy, P.G., Minocha, H.C., Fenwick, B.W., Baker, P.E., Davis, W.C., Blecha, F., 1990. Adjuvanticity of recombinant bovine interleukin-1 beta: influence on immunity, infection, and latency in a bovine herpesvirus-1 infection. Lymphokine Res. 9, 295–307.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sambrook, J., Frisch, E.F., Maniatis, T., 1989. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Scapigliati, G., Buonocore, F., Bird, S., Zou, J., Pelegrin, P., Falasca, C., Prugnoli, D., Secombes, C.J., 2001. Phylogeny of cytokines: molecular cloning and expression analysis of sea bass *Dicentrarchus labrax* interleukin-1beta. Fish Shellfish Immunol. 11, 711–726.

Secombes, C., Zou, J., Daniels, G., Cunningham, C., Koussounadis, A., Kemp, G., 1998. Rainbow trout cytokine and cytokine receptor genes. Immunol. Rev. 166, 333–340.

Serapion, J., Kucuktas, H., Feng, J., Liu, Z., 2004. Bioinformatic mining of type I microsatellites from expressed sequence tags of channel catfish (*Ictalurus punctatus*). Mar. Biotechnol. 6, 364–377.

Shen, L., Stuge, T.B., Zhou, H., Khayat, M., Barker, K.S., Quiniou, S.M., Wilson, M., Bengten, E., Chinchar, V.G., Clem, L.W., Miller, N.W., 2002. Channel catfish cytotoxic cells: a mini-review. Dev. Comp. Immunol. 26, 141–149.

Smith, D.E., Renshaw, B.R., Ketchem, R.R., Kubin, M., Garka, K.E., Sims, J.E., 2000. Four new members expand the interleukin-1 superfamily. J. Biol. Chem. 275, 1169.

Stuge, T.B., Wilson, M.R., Zhou, H., Barker, K.S., Bengtén, E., Chinchar, V.G., Miller, N.W., Clem, L.W., 2000. Development and analysis of various clonal alloantigen-dependent cytotoxic cell lines from channel catfish. J. Immunol. 164, 2971–2977.

Vandenbroeck, K., Fiten, P., Opdenakker, G., Billiau, A., 1994. Two distinct interleukin-1 beta genes in the pig genome. Immunogenetics 40, 82.

Wang, Q., Wang, Y., Xu, P., Liu, Z.J., 2006. NK-lysin of channel catfish: gene triplication, sequence variation, and expression analysis. Mol. Immunol., in press.

Wang, T., Johnson, N., Zou, J., Bols, N., Secombes, C.J., 2004. Sequencing and expression of the second allele of the interleukin-1 beta gene in rainbow trout (*Oncorhynchus mykiss*): identification of a novel SINE in the third intron. Fish Shellfish Immunol. 16, 335–358.

Wang, T., Zou, J., Cunningham, C., Secombes, C.J., 2002. Cloning and functional characterisation of the interleukin-1 beta 1 promoter of rainbow trout (*Oncorhynchus mykiss*). Biochim. Biophys. Acta 1575, 108–116.

Weining, K.C., Sick, C., Kaspers, B., Staeheli, P., 1998. A chicken homolog of mammalian interleukin-1 beta: cDNA cloning and purification of active recombinant protein. Eur. J. Biochem. 258, 994–1000.

Xu, P., Bao, B., He, Q., Peatman, E., He, C., Liu, Z.J., 2005. Characterization and expression analysis of bactericidal permeability-increasing protein (BPI) antimicrobial peptide gene from channel catfish *Ictalurus punctatus*. Dev. Comp. Immunol. 29, 865–878.

Zou, J., Bird, S., Minter, R., Horton, J., Cunningham, C., Secombes, C.J., 2000. Molecular cloning of the gene for interleukin-1beta from *Xenopus laevis* and analysis of expression in vivo and in vitro. Immunogenetics 51, 332–338.

Zou, J., Cunningham, C., Secombes, C.J., 1999. The rainbow trout *Oncorhynchus mykiss* interleukin-1 beta gene has a differ organization to mammals and undergoes incomplete splicing. Eur. J. Biochem. 259, 901–908.