First Study on Interferon Regulatory Factor in Sturgeon: Expression Pattern of Interferon Regulatory Factor in Dabry's Sturgeon *Acipenser dabryanus*

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Interferon regulatory factors (*IRFs*) are crucial regulators in initiating the host innate immune response against pathogen invasions. Dabry's sturgeon (*Acipenser dabryanus*) is particularly a valuable fish species found in the Yangtze River, China for which there is scarce immunological data. In the present study, we investigated the expression profile of sturgeon *IRF* genes. All *adIRFs* were composed of 8 exons and 7 introns, except *adIRF1*, which possessed 9 exons interrupted by 8 introns. Moreover, the predicted protein sequence has a DNA-binding domain (DBD) sharing high identity with spotted gar (*Lepisosteus oculatus*). Regarding the expression patterns, 5 *adIRF* genes were found to be constitutively expressed in all tissues examined, and were significantly higher in lymphoid organs (eg, blood, kidney, intestine, and spleen). Following *Aeromonas hydrophila* infection, the expression of *adIRF1* and *adIRF3* were upregulated in the spleen and caudal kidney, while both the *adIRF5* and *adIRF8* genes were downregulated in caudal kidney. In addition, *adIRF4* was significantly upregulated at 3 h postinfection by *A. hydrophila* in the spleen and caudal kidney. These results suggest that *adIRFs* are related to the immune response to bacterial infection, which will help clarify the function of these *IRFs* and provide a fundamental basis for protecting the Dabry's sturgeon.

Keywords: Dabry's sturgeon (*Acipenser dabryanus*), interferon regulatory factors (*IRFs*), expression

Introduction

SINCE THE FIRST interferon regulatory factor (*IRF1*) was identified as a protein which binds to the *cis*-acting DNA elements of the *IFN-β* gene, 9 *IRF* family members have been identified in mammals, 10 in birds, and 11 in fish (Huang and others 2010, 2015; Feng and others 2015). Previous research has found that *IRFs* are transcription mediators of virus-, bacteria-, and *IFN*-induced signaling pathways and thus play a critical role in antiviral defense, the immune response, as well as the regulation of cell growth and apoptosis (Paun and Pitha 2007). Structurally, all members of the *IRF* family contain a significant homology DNA-binding domain (DBD) in the N-terminal constituted by the first 115–120 amino acids, displaying a unique helix-turn-helix structure (Escalante and others 1998; Battistini 2009). Furthermore, each *IRF* possesses a characteristic *IRF* association domain (IAD) at the carboxyl terminus which mediates their interactions with other proteins to form transcriptional complexes

(Yabu and others 1998). Remarkably, 2 types of association modules have been identified within the IAD domain: (1) IAD1, which is conserved in all *IRFs* but *IRF1* and *IRF2*; and (2) IAD2, which is found only in *IRF1* and *IRF2* (Mamane and others 1999; Taniguchi and others 2001; Savitsky and others 2010). In addition, *IRF* proteins also contain a variety of functional domains, such as the transcription activation domain, transcriptional repression domain, constitutive activation domain, virus activation domain, serine-rich structure domain, and nuclear localization signal (Hiscott 2007; Jia and Guo 2008).

IRF proteins can be divided into 3 categories based on functionality: (1) transcriptional activators (eg, *IRF1*, *IRF3*, *IRF5*, *IRF9*, and *IRF10*); (2) repressors (eg, *IRF8*); and (3) some (eg, *IRF2*, *IRF4*, and *IRF7*) also exert both functions (Huang and others 2010). For instance, *IRF1* of zebrafish (*Danio rerio*) can bind to several *IFN* promoters through various mechanisms and selectively activate *IFNs* via myeloid differentiation factor 88- (*MyD88*-) dependent

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signaling pathways (Liao and others 2016); *IRF3*, in some cases, cooperates with *IRF1* to activate transcription (Savitsky and others 2010); *IRF4* mediates the immune response by activating the expression of other genes during cell differentiation (Ai and others 2017). On the contrary, *IRF4* competes with *IRF5* binding to *MyD88*, thereby negatively regulating toll-like receptor (TLR)-mediated inflammatory responses (Ai and others 2017); however, *IRF5* positively regulates TLR-dependent signaling when bound to *MyD88* (Negishi and others 2005).

Dabry's sturgeon (*Acipenser dabryanus*), is particularly a valuable fish species found in the Yangtze River, China and has been added to "the list of endangered wild animals" by the United Nations. To date, there are no existing reports of *IRFs* in Dabry's sturgeon; however, research into the interferon system of Dabry's sturgeon is significant for both disease prevention and treatment, as well as research and

development into novel drugs. Consequently, the genomic structure and the tissue distribution of (*ad*)*IRF1*, *IRF3*, *IRF4*, *IRF5*, and *IRF8* genes in Dabry's sturgeon were performed in this study. Moreover, we assessed the expression pattern of the *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* genes following *Aeromonas hydrophila* challenge.

Materials and Methods

Experimental fish and bacterium

Dabry's sturgeon (*A. dabryanus*) juveniles (32–37 g) were donated by the Yangtze River Fisheries Research Institute (YFI), Chinese Academy of Fishery Sciences. The fish were maintained in aerated water tanks at 25°C for 1 week to ensure that the animals were healthy before sampling. A virulent strain of *A. hydrophila* named H2 (GenBank Accession NO:

TABLE 1. PRIMERS USED FOR cDNA AND GENOMIC DNA CLONE

Primer name	Sequence (5'-3')	Usage
IRF1-YZ-F/R	CCTGTTTCAAGAACGCGCATG/CTGCAGGCAGGCAACGGGT	cDNA cloning
IRF3-YZ-F/R	ACTTCATCGAAACCCCTCAT/GCTCGTGTCATACTCTCATA	cDNA cloning
IRF4-YZ-F/R	ATGAATTTAGACAGTGACTGCAGC/TTCTTGATGGAAGGGTGCA	cDNA cloning
IRF5-YZ-F/R	ATGAGTCTCCAGCCACGACG/GTGGAGGTCAGGTTGCTGCA	cDNA cloning
IRF8-YZ-F/R	ATGACTGACAGAAACCCTGGTG/TACAGTAATCTGCTGAATTTCACG	cDNA cloning
IRF1-intron-F1/R1	CCTGTTTCAAGAACGCGCATG/AAGCGTCTTTGTCCAAGTCCAT	First intron cloning
IRF1-intron-F2/R2	ACGCTTGCCTTTTCAAACAGTG/TGTCTGGGAGAGAGTTCA TTGC	Second intron cloning
IRF1-intron-F3/R3	GACTGTAACAAACCAGACCCGA/CTCCATTCTGAGGGCACA CTAA	Third, fourth, and fifth intron cloning
IRF1-intron-F4/R4	CCAAGTGTCCCCCTTACCCA/CTGCAGGCAGGCAACGGGT	Sixth, seventh, and eighth intron cloning
IRF3-intron-F1/R1	ACTTCATCGAAACCCCTCAT/ACGACCGCTCGCTACCGC	First intron cloning
IRF3-intron-F2/R2	GGGCGGTAGCGAGCGGTC/TCCACTTCAGGTTTCATAACGAG	Second intron cloning
IRF3-intron-F3/R3	CTCGTTATGAACCTGAAGTGGA/AAGTGAGTGGTCAGTCTG TGTG	Third, fourth, and fifth intron cloning
IRF3-intron-F4/R4	ACCCCTCCCCCTTGCTTGATA/GCTCGTGTCATACTCTCATA	Sixth and seventh intron cloning
IRF4-intron-F1/R1	AGTGGCTGATTGAACAGATTGAC/TGACTTCTTTCCACCATT TCCT	First intron cloning
IRF4-intron-F2/R2	AATGACTTTGAGGAAATGGTGA/TAGTGTAGCCAAGCGAG TTCATA	Second intron cloning
IRF4-intron-F3/R3	TGAACCTCGCTTGGCTACACTATG/GTAAGGCATTTTCAGAGTG AGGCT	Third intron cloning
IRF4-intron-F4/R4	CGTAACTGGAGGGAGTATGAGCA/GAAACGGGTAGGGGAAA ATGAC	Fourth and fifth intron cloning
IRF4-intron-F5/R5	GTCATTTTCCCCTACCCGTTTC/TTCTTGATGGAAGGGTGCA	Sixth and seventh intron cloning
IRF5-intron-F1/R1	ATGAGTCTCCAGCCACGACG/CGCAGGTTTCGCTTTTCCACTT	First intron cloning
IRF5-intron-F2/R2	AAGTGGAAGCGAACCTGCG/GTATGTTCTGGCAAAGGGGA AGTT	Second, third, and fourth intron cloning
IRF5-intron-F3/R3	AACCTCCCTTTGCCAGAACATAC/CACTGGCAAAGGCGTA TGGCAT	Fifth intron cloning
IRF5-intron-F4/R4	ATGCCATACGCCTTTGCCAGTG/CACTGCCCTTGAGTCTGT TGGTTC	Sixth and seventh intron cloning
IRF8-intron-F1/R1	TGTGGGAAAATGAAGAGAAAACC/TGTTCAAAGCACAGCG AAGCCTC	First intron cloning
IRF8-intron-F2/R2	GCTTTGAACAAGAGCCCCGACT/GCAGGGGGGAACCTTCA CATT	Second, third, fourth, and fifth intron cloning
IRF8-intron-F3/R3	CGCAGTAACCGTGAGGGCAT/TACAGTAATCTGCTGAAT TTCACG	Sixth and seventh intron cloning

MF399208) was isolated and identified from diseased Chinese sturgeon (*A. sinensis*) in a previous study and was stored in YFI.

Cloning of cDNA and genomic DNA sequences

cDNA samples were prepared from the head kidney with the First-Strand cDNA Synthesis Kit (Fermentas, Canada). DNA samples were isolated from the muscle using a standard phenol/chloroform extraction method. First, degenerate primers (Table 1) were used for PCR amplification of the internal region of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* obtained from the cDNA samples. PCR products were respectively isolated using a Gel Extraction Kit (Tiagen, China), cloned into a pMD18-T vector (TaKaRa, Japan), and transformed into *Escherichia coli* strain DH5 α competent cells. The putative clones were then screened via PCR using the aforementioned primers, and the selected clones were sequenced.

Sequence analysis

First, the open reading frame (ORF) of the genes was predicted using ORF Finder (www.bioinformatics.org/sms/orf_finder.html). The prediction of the amino acid sequences was performed using a translate program in ExPASy (<http://web.expasy.org/translate>). The physicochemical properties of the deduced proteins were analyzed via ProtParam (<http://web.expasy.org/protparam>). The exon-intron structure was determined by aligning the cDNA sequences to the genomic DNA sequence using the web-based tool, Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). The phylogenetic tree was constructed using amino acid multiple alignment and the neighbor-joining method within the MEGA6 program with the JTT matrix-based model. The degree of confidence for each branch point was determined by bootstrap analysis (1,000 times).

Tissue distribution and immune challenges

To examine the expression pattern in different tissues, 9 healthy Dabry's sturgeon were sacrificed, and 12 tissues (gills, skin, muscle, liver, spleen, head kidney, caudal kidney, intestine, eye, brain, heart, and blood) were removed for RNA extraction. To evaluate the immune responses of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8*, the fish held at 25°C were randomly divided into 2 groups: one group was intraperitoneally injected with 200 μ L *A. hydrophila* suspension with PBS (1.9×10^9 cfu/mL), and the other group was intraperitoneally injected with 200 μ L PBS (0.1 mol/mL, pH = 7.2) per gram body weight as a control. Four individuals were sampled at 3, 12, 24, and 36 h postinjection (hpi) on each occasion, with the caudal kidney and spleen removed, using

FIG. 1. PCR amplification by primers of *Aeromonas hydrophila* virulence gene. M: DL2000DNA Marker; 1: PCR product; 2: positive control; 3: negative control.

the primers of *A. hydrophila* virulence gene (Ai and others, 2015) to verify the disease caused by H2 (Fig. 1).

Real-time quantitative polymerase chain reaction

The tissues and organs described earlier were used for total RNA extraction, and the cDNA samples were prepared using the First-Strand cDNA Synthesis Kit (Fermentas, Canada). The specific primers were designed to detect the corresponding gene expression (Table 2) and pretested to ensure that each primer pair could not amplify the genomic DNA using real-time quantitative polymerase chain reaction (qRT-PCR). The KAPA SYBR[®] FAST qPCR Master Mix (KAPA BIOSYSTEMS) and a Step-one Plus real-time PCR system (ABI) were used to measure the expression of *adIRFs* as described previously (Xu and others, 2010; 2014a, 2014b). Dabry's sturgeon β -actin was amplified with the same qRT-PCR temperature profile for use as the internal reference. The total reaction mixture and reaction procedure for qRT-PCR were performed as described previously (Xu and others 2010, 2014a).

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software. A one way-analysis of variance (ANOVA) followed

TABLE 2. PRIMERS USED FOR REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION

Primer name	Sequence (5'-3')	GenBank accession No.
IRF1	CCACAGCCGACAGCACAAAC/TCAGGAAACCTTTGCCATTA	KY624489
IRF3	ACCCCTCCCCCTTGCTTGATA/GGTTGGTGTGTAAATCTCCG	KY624490
IRF4	AGTGGCTGATTGAACAGATTGAC/TGACTTCTTTCCACCATTTCCCT	KY624491
IRF5	TGTATCAGAAAGGAGAACTAACG/CACCTGCCCTTGAGTCTGTTGGTTC	KY624492
IRF8	GTCTTAGGCAGTGGCTGATTGAG/GATTTCTGGTTGTAGTCCTGTT	KY624493
β -actin	CCTTCTTGGGTATGGAATCTTGC/CAGAGTATTTACGCTCAGGTGGG	MF536662

TABLE 3. THE PHYSICOCHEMICAL PROPERTIES OF DABRY'S STURGEON *IRF*S PROTEIN

Features	<i>adIRF1</i>	<i>adIRF3</i>	<i>adIRF4</i>	<i>adIRF5</i>	<i>adIRF8</i>
ORF (bp)	978	1335	1341	1356	1299
Molecular weight (kilodalton, kDa)	79808.31	49764.92	51683.46	56597.35	49901.32
Theoretical pI	5.06	5.27	6.21	5.41	5.88

by the LSD *post hoc* test was used to analyze the induction expression data. Differences were considered to be significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively.

Results

Molecular cloning and characterization of *adIRFs*

In this study, we cloned the ORF of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* that consist of 978 bp, 1,335 bp, 1,341 bp, 1,356 bp, and 1,299 bp, respectively. Each cDNA sequence encodes a protein composed of 316, 445, 447, 452, and 433 amino acids residues, respectively, and the physicochemical properties of the proteins encoded by these 5 *adIRF* genes have been summarized in Table 3.

Similar to the *IRF* genes of other vertebrates, the deduced amino acid sequences of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* contain the DBD in the N-terminal, which is characterized by 5 tryptophan repeats. The sequence of the putative *adIRF* protein is highly similar to that of other species, ranging from 54.0% to 94.8% (Table 4); however, the most similar species to possess the DBD domain in the 5 *adIRF* proteins is spotted gar, with 78.6%, 67.3%, 92.0%, 88.7%, and 94.8% sequence identity, respectively (Table 4).

To verify the 5 *adIRF* genes, a phylogenetic tree was constructed (Fig. 2). The *IRF* family can be divided into 4 subfamilies: (1) *IRF1* subfamilies (contains *IRF1* and *IRF2*); (2) *IRF3* subfamilies (contains *IRF3* and *IRF7*); (3) *IRF4* subfamilies (contains *IRF4*, *IRF8*, *IRF9*, and *IRF10*); and (4) *IRF5* subfamilies (contains *IRF5* and *IRF6*). It is apparent that *adIRF1* and *adIRF5* were clustered in close proximity to *Polyodon spathula* *IRF1* and *IRF5*, respectively, followed by those of other fish species. Although *adIRF4* and *adIRF8* were not clustered extremely close to *P. spathula*, they were closely related to spotted gar (*Lepisosteus oculatus*) *IRF4* and *IRF8*, respectively. Moreover, *adIRF3* was found to be equally distant to the *IRF3* of both spotted gar and tilapia (*Oreochromis spp*) (Fig. 2).

Genomic structure analysis of *adIRFs*

The genomic DNA and cDNA sequences were aligned using Clustal Omega. The *adIRF1* gene contained a 9 exon and 8 intron structure, while that of *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* consisted of 8 exons interrupted by 7 introns (Fig. 3). An intron phase analysis revealed that the classes of *adIRF1* introns 1, 4, and 7 to be in phase 0, leaving the others

TABLE 4. IDENTITY COMPARISON BETWEEN TELEOST INTERFERON REGULATORY FACTOR PROTEINS

Molecule	Species	Identity (%)					
		DBD			Whole length		
		Human	Spotted gar	Dabry's sturgeon	Human	Spotted gar	Dabry's sturgeon
IRF1	Dabry's sturgeon	71.4	76.8		39.0	43.7	
	Spotted gar	82.3		76.8	44.1		43.7
	Frog	85.8	77.0	70.5	53.3	43.0	36.3
	Chicken	91.2	82.3	75.0	59.4	44.7	39.3
	Human		82.3	71.4		44.1	39.0
IRF3	Dabry's sturgeon	54.0	67.3		33.3	46.1	
	Spotted gar	47.8		67.3	34.2		46.1
	Frog	51.3	50.4	54.0	31.1	33.3	35.7
	Human		47.8	54.0		34.2	33.3
IRF4	Dabry's sturgeon	77.0	92.0				
	Spotted gar	83.2		92.0	67.3	81.4	
	Frog	84.1	85.8	81.4	70.6		81.4
	Chicken	91.7	89.9	81.7	83.6	71.5	69.0
	Human		83.2	77.0		70.6	67.3
IRF5	Dabry's sturgeon	68.1	88.7		54.3	75.7	
	Spotted gar	71.7		88.7	54.6		75.7
	Frog	69.9	74.5	76.1	58.6	53.8	56.3
	Chicken	64.7	63.2	61.1	57.0	50.4	49.8
	Human		71.7	68.1		54.6	54.3
IRF8	Dabry's sturgeon	88.6	94.8		60.6	80.8	
	Spotted gar	90.4		94.8	62.4		80.8
	Chicken	97.4	89.5	89.5	73.9	60.7	60.2
	Human		90.4	88.6		62.4	60.6

DBD, DNA-binding domain.

in phase 1 (Fig. 3). In addition, the first, third, and last introns of *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* were in phase 0, while all other introns were in phase 1 (Fig. 3).

Tissue distribution

To examine the tissue distribution pattern of *IRF1*, *IRF3*, *IRF4*, *IRF5*, and *IRF8* in Dabry's sturgeon, the *in vivo* expression level of these *adIRFs* were quantified using real-time PCR. The results revealed that these 5 genes were constitutively expressed in all 12 tissues obtained from healthy fish (Fig. 4). In addition, all these genes were highly expressed in the blood, especially *adIRF1*. Similar to *adIRF1*, *adIRF5* appeared to exhibit a high level of expression in the blood, followed by the skin, rather than the head kidney; however, *adIRF1*, *adIRF3*, *adIRF4*, and *adIRF8* were expressed at moderate or very low levels. *adIRF3* was highly expressed in all tissues. In the *adIRF4* subfamily, *adIRF4* was highly expressed in the blood, spleen, caudal kidney, and heart, while *adIRF8* was predominantly detected in the liver, followed by the spleen and caudal kidney.

Gene expression of *adIRFs* in response to *A. hydrophila* challenge

To further investigate the different patterns of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8*, the expression level induced in response to *A. hydrophila* challenge was examined. We found that *A. hydrophila* stimulation upregulated *adIRF1* expression in the spleen (3.11-fold; $P < 0.05$) and the caudal kidney (7.63-fold; $P < 0.01$) tissue at 36 hpi and 3 hpi, respectively (Fig. 5). Similarly, the expression of *adIRF3* was found to be upregulated in the spleen at 3 hpi (3.00-fold; $P < 0.05$) and 24 hpi (32.65-fold; $P < 0.01$), as well as the caudal kidney at 36 hpi (4.07-fold; $P < 0.01$). While the expression of both *adIRF5* and *adIRF8* genes were downregulated in the caudal kidney at 24 hpi ($P < 0.05$), it was not affected or increased in the spleen following treatment with *A. hydrophila* (Fig. 5). For *adIRF4*, the expression was significantly upregulated at 3 hpi following *A. hydrophila* exposure in the spleen ($P < 0.01$) and caudal kidney ($P < 0.01$) (Fig. 5).

Discussion

IRFs are critical regulators of the immune response and immune cell development, and abnormalities in *IRF* expression and function have been increasingly linked to numerous diseases (Zhao and others 2015). Further information on the *IRFs* from lower vertebrates will aid our understanding of the mechanisms of innate and adaptive immunity. In this study,

FIG. 2. A neighbor-joining phylogenetic tree of vertebrate *IRF* members. The tree was constructed using an amino acid multiple alignment and the neighbor-joining method within the MEGA6 program (Tamura and others, 2008). The evolutionary history was inferred by using the method based on the JTT matrix-based model. The percentage of trees in which the associated taxa clustered together is shown next to the branches based on 1,000 bootstrap. The accession number for each sequence was given after the common species name and molecular type. The Dabry's sturgeon *IRF* genes are shown in bold.

FIG. 3. Schematic diagrams of exon–intron structure of *adIRF* genes in Dabry’s sturgeon. Boxes represent exons, and horizontal lines connecting exons represent introns. The number in the box represents the nucleotide length (base pairs). The intron phase is indicated under the bar.

we identified and characterized the structure, mRNA tissue distribution, and gene expression of *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* from Dabry’s sturgeon. Using PCR-based techniques, the cDNA and genomic DNA sequences were obtained.

Phylogenetically, all of the investigated genes were clustered into one group with the corresponding genes from other teleosts, and were found to share the closest relationship with *P. spathula* (*IRF1* and *IRF5*) and spotted gar (*IRF3*, *IRF4* and *IRF8*). Structurally, similar to other species, *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* contain a DBD in the N-terminus. The DBD is typical of all *IRF* members, which forms a helix-turn-helix structure responsible for binding to the interferon-stimulated response element (IRSE)/interferon regulatory factor-binding element (IRF-E) consensus in the target promoters (Xia and others 2012). The DBD domain of *adIRFs* share high sequence similarity with other species, ranging from 54.0% to 94.8%,

with 67.3% to 94.8% shared with the spotted gar homologs. Furthermore, *adIRF1* exhibited a 9 exon and 8 intron structure, while *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* consisted of 8 exons interrupted by 7 introns. The intron phases in *adIRF1* consisted of introns 1, 4, and 7 in phase 0, introns 2, 3, 5, 6, and 8 in phase 1, and were well conserved in *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* with introns 1, 3, and 7 in phase 0, and introns 2, 4, 5, and 6 in phase 1. In previous studies, Huang and others (2010) indicated that *IRF1* all had a 9 exon and 8 intron gene structure, except for zebrafish, for which only 8 exons were present; however, the number of exon–introns in *IRF2* to *IRF10* is not highly conserved from teleosts to mammals.

The tissue distribution analysis revealed that the *adIRFs* were strongly transcribed in lymphomyeloid-rich tissues (eg, spleen, head kidney, caudal kidney, liver, blood, and intestine) in Dabry’s sturgeon. The *adIRF1* expression pattern was high in blood, head kidney, caudal kidney, intestine, and

FIG. 4. Tissue distribution of *IRF* in Dabry’s sturgeon. I, SP, BR, HK, M, L, E, SK, G, CK, BL, and H represent the intestine, spleen, brain, head kidney, muscle, liver, eye, skin, gill, caudal kidney, blood, and heart, respectively.

spleen and is similar to the *IRF1* expression patterns in mammalian and other fish species (Sun and others 2007; Shi and others 2010; Liu and others 2011; Xu and others 2015; Zhan and others 2016), with predominant expression in immune-related tissues. Unlike the expression profiles of mammalian *IRF3* (Honda and Taniguchi 2006), our study demonstrates that *adIRF3* was particularly expressed in the immune tissues where the lymphoid and myeloid cells are abundant; this finding is consistent with turbot, Japanese flounder, and *Gadus macrocephalus* (Hu and others 2011a, 2011b; Sun and others 2015). In previous studies, *IRF4*, known to be essential for the development and functionality of T and B cells, has been reported to be expressed only in lymphocytes (Marecki and others 1999; Holland and others 2010; Bathige and others 2012; Ai and others 2017). Nevertheless, *IRF4* and *IRF8* were strongly detected in both hematopoietic and lymphoid tissues, as well as some non-immune tissues of Dabry's sturgeon. *adIRF5* is highly expressed in the blood, head kidney, caudal kidney, and skin which exhibits a similar expression pattern to that observed for grass carp *IRF5* (Xu and others 2010a), and turbot *IRF5* (Xia and others 2012).

A. hydrophila is a gram-negative bacterium, which causes a hemorrhagic disease in most fish. To obtain further information regarding the role of *adIRF* genes for the treatment of bacterial infections, the expression pattern of *adIRF* genes following *A. hydrophila* challenge was performed in the present study. The results show that exposure to *A. hydrophila* upregulated the expression of both *adIRF1* and *adIRF3*. In addition, *IRF1* expression is known to be sensitive to viral and bacterial challenge (Yao and others 2010; Lu and others 2014; Xu and others 2015; Zhan and others 2016). In large yellow croaker (*Pseudosciaena crocea*), the expression profiles of *IRF1* in blood, spleen, and liver increased after an injection of poly I:C (Yao and others 2010). Similarly, *Vibrio anguillarum* and *A. hydrophila* induced the upregulation of *IRF1* expression in the blood, liver, kidney, and spleen of half-smooth tongue sole (*Cynoglossus semilaevis*) and blunt snout bream (Lu and others 2014; Zhan and others 2016), which is consistent with the findings for *adIRF1*. Although the expression changes vary in magnitude among different fish species, the *IRF* gene expression pattern exhibits a similar profile in the spleen and kidney. *IRF3* in large yellow croaker did not demonstrate significant changes following *V. parahaemolyticus* injection; however, *IRF3* expression was significantly increased following an injection of LPS (Yao and others 2012). Thus, the authors considered that the differential levels of *IRF3* expression in large yellow croaker between the LPS and bacteria injection groups might be attributed to the LPS content in the bacteria being too low to induce an immune response (Yao and others 2012). The upregulation of *adIRF3* in response to *A. hydrophila* challenge in the present study is similar to that exhibited by *IRF3* in the Asian swamp eel following infection with *A. hydrophila* (Zhan and others 2016). Therefore, we considered that the reason for this result may be attributed to a number of factors, including different bacterial concentrations and individual variability in the immune response. Although both *IRF4* and *IRF8* are members of the *IRF4* subfamily, the expression patterns of *adIRF4* and *adIRF8* are not identical following *A. hydrophila* stimulation. In the current study, *A. hydrophila*

challenge led to significant upregulation of *adIRF4* in the caudal kidney and spleen; however, *A. hydrophila* downregulated *adIRF8* expression in the caudal kidney. These findings indicate that *adIRF4* and *adIRF8* may play diverse roles in the antibacterial response, despite belonging to the same *IRF* subfamily. In rock bream, changes in *IRF4* expression (upregulation or downregulation) began 3 h after *E. tarada* infection (Bathige and others 2012). Moreover, GCRV upregulated zebrafish *IRF4a* from 3 hpi, and SVCV upregulated zebrafish *IRF4a* from 6 hpi (Ai and others 2017). In addition, the expression of Asian swamp eel *IRF4* was upregulated within 12 h following *A. hydrophila* infection, and lasted for up to 48 h (Xu and others 2014b). These findings indicate the *IRF4* function in the early stage of bacterial or viral infection. In mammals, *IRF5* is a key regulator of *IFN- α* and *IFN- β* expression, and the associated immune reaction is virus specific (Barnes and others 2001; Tamura and others 2008); however, in teleosts, the expression of *IRF5* is only induced by both viruses and bacteria (Xu and others 2010b, 2015). In contrast to Grass carp (Xu and others 2010a) and Asian swamp eel *IRF5* (Xu and others 2015), which are upregulated by *Flavobacterium columnare* and *A. hydrophila*, respectively, stimulation with *A. hydrophila* downregulated *adIRF5* in the caudal kidney at 24 hpi. In addition, *A. hydrophila* upregulated Asian swamp eel *IRF5* *in vivo* but induced little change *in vitro* (Xu and others 2015), suggesting that *IRF5* may play different roles depending on the species or conditions.

In conclusion, we identified and characterized the structure, mRNA distribution, and gene expression of the *adIRF1*, *adIRF3*, *adIRF4*, *adIRF5*, and *adIRF8* genes in this study. Five *adIRFs* were constitutively expressed in all 12 tissues from Dabry's sturgeon and exhibited particularly high expression in the immune tissues where the lymphoid and myeloid cells are abundant. While *adIRF1* and *adIRF3* were significantly upregulated *in vivo* following *A. hydrophila* infection, *adIRF5* and *adIRF8* were downregulated in the caudal kidney. Moreover, *adIRF4* is upregulated in response to *A. hydrophila* challenge in both the spleen and caudal kidney. The results acquired in this study will provide a basis for elucidating the innate immune mechanisms in teleosts.

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Author Disclosure Statement

No competing financial interests exist.

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