Expression and biological activity of two types of interferon genes in medaka (Oryzias latipes)

Shun Maekawa a, Yi-An Chiang a, Jun-ichi Hikima b, Masahiro Sakai b, Chu-Fang Lo c, Han-Ching Wang a,⁎, Takashi Aoki a,⁎⁎

⁎ Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan
⁎⁎ Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki 889-2192, Japan

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A B S T R A C T
Type I interferon (IFN) is one of most important cytokines for antiviral responses in fish innate immunity, after the induction pathway following pattern recognition. In this study, 2 types of type I IFN mRNA from a medaka (Japanese rice fish; Oryzias latipes) were identified and classified (phylogenetic analysis) into subgroup-a and -d by (designated olIFNa and olIFNd, respectively). Both olIFNa and olIFNd (encoding 197 and 187 amino acid residues, respectively) contained 2 cysteines. Gene expression pattern of olIFNa, olIFNd and IFN-stimulated genes (ISGs) was assessed (quantitative real-time reverse transcriptase PCR, qRT-PCR) in various organs (i.e., whole kidney, liver and spleen) of medaka stimulated by polyI:C or infected with nervous necrosis virus (NNV). Expression of olIFNa, olIFNd and ISGs, especially the ISG15 gene, were significantly upregulated after NNV-infection. Furthermore, olIFNa, olIFNd and ISGs mRNAs were sufficiently induced in DIT cells (i.e., medaka hepatoma cell line) transfected with polyI:C or infected with NNV. In addition, in vitro biological activities of recombinant olIFNa and olIFNd (rollIFNa and rollIFNd) produced by mammalian cell line HEK293T were also characterized. Expression of GIG1a and ISG15 genes in kidney cells of adult medaka were induced by rollIFNa or rollIFNd. The olIFNs-overexpressing DIT cells had reduced viral titers following NNV infection. Therefore, we inferred that 2 type I IFNs were involved in innate immunity (antiviral response) in medaka fish.

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1. Introduction
Type I interferon (IFN) is among the most important cytokines for antiviral responses in innate immunity. Mammalian type I IFNs constitute a multigene family grouped into IFN-α, IFN-β, IFN-δ, IFN-ε, and IFN-ω [1,5,6,7]. Mammalian type I IFN genes do not contain introns and are closely clustered within the same genome. Expression of type I IFN is induced by pattern recognition receptors (PRRs), e.g., toll-like receptor (TLR)s, RIG-I-like receptors (RLRs) and cytosolic DNA sensors (CDSS). There are 3 RLRs have 3 members, namely RIG-1, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2); they are localized in intracellular vesicles and recognize double strand RNA (dsRNA) of virus genome and poly(C) (dsRNA analog). Activated RLRs induces transcription of type I IFN, by activating signal cascades of mediator of IRF3 activation (MITA), TANK-binding kinase 1 (TBK1) and IFN regulatory factors (IRFs) 3 and 7[2,3]. The secreted type I IFN binds with IFN-receptor (IFNR)-1 and IFNR-2, which delivers a signal through the JAK/STAT pathway. As a consequence, STAT associates with IRF9 to form a heterotrimeric complex (IFN-stimulated gene factor 3, ISGF3), which binds to the upstream IFN-stimulated response elements (ISRE) and activates transcription of various IFN-induced genes to promote antiviral activity, including production of inflammasome proteins [4].

Teleost type I IFN genes have been identified in various fish species. Teleost type I IFN genes retain the gene organization of 5 exons and 4 introns, unlike in mammals [5,6]. The number of type I IFN genes varies among teleost fish species. There are 4 type I IFN genes in zebrafish, Danio rerio [7–9], 5 in rainbow trout (Onco-rhynchus mykiss) [7,10,11] and 11 in Atlantic salmon (Salmo salar)
[6,12,13]. Meanwhile, Japanese pufferfish (Takifugu rubripes) and medaka (Oryzias latipes) each have only a single type I IFN gene [7]. Based on the cysteine patterns in the mature peptide, teleost type I IFNs could be divided into 2 major groups, with either 2 or 4 cysteine residues (2C or 4C groups, respectively). Phylogenetically, teleost type I IFNs are also classified into 4 subgroups, denoted IFNa, -b, -c, and -d, with IFNa and -d constituting the 2C type I IFNs and IFNb and -c the 4C type I IFNs [6,11]. In zebrafish, type I IFNs of the 2-cysteine group (IFNα1 and -4) bound to the cytokine receptor family B (CRFB1-4, CRFB5 complex, whereas the CRFB5 chain associated with CRFB2 to form the receptor for 4-cysteine IFNα2 and -3 [9]. Although IFNα1-3 had an anti-viral effect, IFNα4 (subgroup d type I IFN) did not have [9]. In a salmon study, IFNa and -c had less active, and IFNd had no activity [13]. Antiviral activity of IFNd cells [14]. Orange-spotted grouper (Epinephelus fasciatus) IFNs, which belong to subgroup-d, also have important biological functions, including stimulation of Mx expression in primary head kidney cells [14]. Orange-spotted grouper (Epinephelus coioides) and rock bream (Oplegnathus fasciatus) IFNs, which belong to subgroup-d, also have important biological functions, including stimulation of Mx expression [15,16], It was suggested that the IFN subgroups is the only type of IFN in the superorder Acanthopterygii, including Japanese pufferfish, medaka, stickleback and rock bream [5,7,16,17]. However, 4C group and 2C group IFN genes were recently identified in turbot (Scophthalmus maximus) [17]. The 4C group of type I IFN in turbort had clear antiviral activity, whereas the 2C group did not. Consequently, it was suggested that there was another subtype of type I IFN, in addition to IFNd, that has been identified in the superorder Acanthopterygii.

In medaka, a well-characterized experimental model fish, it has been reported that adult fish and some cell lines are susceptible to red-spotted grouper and striped jack nervous necrosis viruses (NNV) [18,19]. However, anti-virus activity though IFN systems in medaka have apparently not been reported. In this study, we report cDNA cloning 2 kinds of type I IFN cDNAs from medaka, designated olIFNa and olIFNd. Furthermore, in vitro biological activity of medaka IFNs were also investigated using recombinant proteins.

2. Materials and methods

2.1. Fish

Cab strain medaka were maintained in several transparent plastic tanks with a water circulating system (28 °C) and under a 14-h light and 10-h dark cycle. In all experiments, fish that were 3–4 month old and weighing 200-300 mg were used. PolyLC was injected in adult medaka (10 μg/g BW injected intraperitoneal, IP). Control group fish were injected with PBS. For NNV challenge, adult medaka were injected IP with 10 μL orange-spotted grouper NNV suspension (108 TCID50/mL).

2.2. Cloning olIFNa and olIFNd cDNA

Two putative sequences of type I IFN genes were identified in the medaka genome database at the Ensembl Genome Database Project (http://asia.ensembl.org/Oryzias_latipes/). Based on the 2 putative sequences, primers were designed to clone the cording regions of olIFNa and olIFNd cDNAs. Total RNA was extracted from various organs using RENZ™ C&T reagent (Protech Technology, Taipei, Taiwan) according to manufacturer’s instructions. Total RNAs were subjected to cDNA synthesis using SuperScript III™ Reverse Transcriptase (Life Technologies, Grand Island, NY, USA) and Anchor dT primer or random primer. The PCR products were cloned into RBC T&A cloning vector (RBC Bioscience) and sequenced. Multiple alignments of IFN amino acid sequences were performed using the ClustalW program (Version 2.1, DDBJ). The putative cleavage site of the signal peptide was identified using the SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/).

2.3. Quantitative real-time reverse transcriptional PCR analysis

Quantitative reverse transcription PCR (qRT-PCR) was performed using KAPA SYBR Fast qPCR Master Mix (Kapa Biosystems, Inc., Wilmington, MA, USA). Gene expression levels were normalized to that of elongation factor 1 alpha (EF1a). The mean threshold cycle was used to determine relative expression levels. Primers used for qRT-PCR were designed using Primer3 software [20]. Primers used for the qRT-PCR study are listed in Table S1.

2.4. Expression of recombinant proteins in HEK293T cells

A modified vector from pFUSE-mlgG2a-Fc (Invitrogen, Carlsbad, CA, USA) was used. A fragment containing a 10-histidine-tag (His-tag) and stop codon was generated by PCR. Sequence encoding mature olIFNa, olIFNd and EGFP peptides were amplified using Ex Taq and cloned into pFUSE-His vector. Sequences of primers used for the plasmid construction are listed in Table S1. Then, HEK293T cells were seeded in a 10-cm culture dish in 10 mL Dulbecco’s Modified Eagle’s Medium (Gibco, Carlsbad, CA, USA) containing 10% fetal calf serum (PAA Laboratory, Pasching, Austria). When cultures reached ~80% confluence, the culture medium was changed to Opti-MEM (Gibco). Then, cells were transfected with 10 μg plasmid vector by adding 15 μL of FuGENE HD Transfection Reagent (Roche Diagnostics, Basel, Switzerland) to each dish containing 500 μL Opti-MEM (Gibco). After 72 h, the culture supernatant containing recombinant His-tag olIFNs and EGFP protein were purified using 2 column chromatography steps, nickel-chelating Sepharose Fast Flow (GE Healthcare BioSciences Co., Pisacataway, NJ, USA) and NAP-5 columns (GE Healthcare BioSciences Co.). Each fraction sample was analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blotting (the latter was performed using anti-histidine polyclonal antibody). Signals were detected using a Western Lightning Plus–ECL chemiluminescence detection kit (Perkin–Elmer, Waltham, MA, USA).

2.5. In vitro culture of DIT cell line

The DIT cell line, derived from medaka hepatoma [21], was obtained from the Riken cell bank and maintained in Leibovitz’s L-15 (Gibco) medium containing 20% FBS (Sigma–Aldrich, St. Louis, MO, USA) and 10 mM HEPEs (pH 7.6) at 33 °C. For transfection, DIT cells were cultured in 24-well plates at a concentration of 1 × 105 cells/well. Before transfection, cells were washed with PBS, and given fresh culture medium. For each well, 50 ng of pFUSE-His vector containing mature peptides region of olIFNs described above were mixed with 0.75 μL of FuGENE HD Transfection Reagent (Roche Diagnostics). The DNA mixtures were transfected to DIT cells in 25 μL of Opti-MEM (Gibco). To determine effects of intracellular polyLC stimulation, 500 ng of polyLC was transfected as described above.

2.6. In vitro culture of renal hematopoietic cells

Kidney marrow cells from adult medaka (1 × 105 cells/well) were cultured in a mixture containing L-15 medium (Gibco), 5% fetal bovine serum (Sigma–Aldrich), and 100 ng/mL of recombinant proteins or 50 μg/mL of polyLC. Cells were incubated at 28 °C.
2.7. Statistics

Error bars represent standard error of the mean (SEM) in all figures. Comparisons between 2 groups were made with Student’s t-test, with \( p < 0.05 \) considered significant.

3. Results

3.1. Identification of type I IFN genes in medaka

At the outset, an in silico search of the medaka genome database was done using the Ensembl genome database project. Thereafter, primers were designed and RT-PCR was done for cloning cDNAs. Two cDNA sequences of type I IFN were identified (Fig. S1). Based on phylogenetic sequence analysis, 1 IFN gene was classified in subgroup a (GenBank accession number LC066594) and another in subgroup d (GenBank accession number LC066595; Fig. 1). Therefore, these 2 IFN genes of medaka were designated oIFNa and oIFNd, respectively.

The oIFNa gene encoded a protein consisting of 197 amino acid residues (Fig. S1A). The SignalP program predicted the secretory signal sequence and a potential cleavage site for a signal peptidase between residues 19 and 20. Although the gene consisted of 4 exons and 3 introns (unlike zebrafish IFNα1, which has 5 exons and 4 introns), predicted IFN receptor 1 and 2 binding sites and 2 cysteine residues were conserved. Furthermore, oIFNa had a consensus sequence for N-glycosylation (Asn- Xaa- Ser/Thr, where Xaa is any amino acid residue except Pro) on Asn 31. The oIFNa peptides shared only 27 and 24% amino acid identity with zebrafish IFNα1 and salmon IFNa, respectively.

The oIFNd gene encoded a protein with 187 amino acid residues (Fig. S1B). The SignalP program predicted the secretory signal sequence and a potential cleavage site for a signal peptidase between residues 17 and 18. Mature polypeptide (170 amino acid residues) of oIFNd peptides was only 37% identical to zebrafish IFNα4, 44% identical to salmon IFN1, and 45% identical to Japanese pufferfish IFN1. Furthermore, based on a domain search, oIFNd had a predicted IFN receptor 1 and 2 binding domains. Moreover, oIFNd had 2 consensus sequences for N-glycosylation on Asn 47 and 50.

\[ \text{Fig. 1. Phylogenetic tree of type I IFN genes in teleost fish and schematic diagram of medaka IFNs structures. (A) In teleost fish, 4 subgroups (a, b, c and d type I IFN) were identified by phylogenetic tree analysis. In this study, oIFNa and oIFNd, included in subgroup-a and -d IFN, respectively, in teleost fish, were identified. (B) Schematic diagram of oIFNa and oIFNd peptides. Gray bar indicates signal peptides. C: cysteine residues. Boxes represent putative IFN receptor binding sites. Bars with circles represent N-linked sugar chains.} \]
3.2. Expression of olIFNa and olIFNd mRNA

It has been reported that adult medaka are susceptible to red-spotted grouper and striped jack nervous necrosis virus (NNV) [19]. However, whether type I IFN genes and ISGs (e.g. Mx, GIG1a, ISG15 and ISG56 genes) could respond to the virus challenge has apparently not been reported. To investigate whether expression level of IFNs mRNA on medaka was enhanced by virus challenge, medaka were subjected to NNV or polyI:C treatment and expression level of olIFNa and olIFNd and IFN-induced genes in the liver, spleen and whole kidney were determined (qRT-PCR; Figs. 2–4).

In the liver, expression levels of Mx, GIG1a, ISG15 and ISG56 genes were upregulated after exposure to polyI:C, compared to the PBS-treated group (Fig. 2A). However, expressions of olIFNa and olIFNd were not induced by polyI:C treatment. Following exposure to NNV, there was increased NNV copy numbers in the liver, compared to the control group (Fig. 2B). Although expression of ISG15 genes was induced at 48 hpi, the level of olIFNa and olIFNd gene expressions did not change in the liver.

Expression of ISGs were significantly upregulated after polyI:C induction in the kidney (Fig. 3A). However, olIFNa and olIFNd genes were not induced by polyI:C. Expression levels of Mx, GIG1a and ISG15 in the kidney were increased at 12 h after NNV-injection. Furthermore, ISG56 gene expression was also induced at 48 hpi. Expression of the olIFNa gene was significantly increased at 12 hpi, whereas olIFNd gene expression was significantly enhanced at 1 and 12 h after NNV injection (Fig. 3B).

In the spleen, expression of Mx and GIG1a genes were upregulated after polyI:C treatment, compared to PBS-treated groups (Fig. 4A). Unlike ISGs, olIFNa and olIFNd genes were not highly expressed in the spleen polyI:C treatment. Expression levels of Mx and GIG1a genes were increased at 1 and 12 h after NNV-injection, although the difference was not significant compared to the control group. Expression of olIFNa and olIFNd genes were induced 6 h after NNV-injection (Fig. 4B).

![Gene expression analysis in the liver of olIFNa, olIFNd and IFN-stimulated genes responding to polyI:C administration. (A) PolyI:C was injected in adult medaka (10 μg/g BW IP) whereas controls were injected with PBS. At 6, 12, 24 and 48 h after injection, fish were killed, the liver collected, RNA extracted, and expression level of specific genes determined by qRT-PCR (n = 4, *p < 0.05 compared to PBS group). (B) Orange spotted grouper NNV was injected IP in adult medaka. At 1, 6, 12 and 48 h after injection, liver were collected, RNA extracted and expression level of each genes and NNV copy numbers determined by qRT-PCR. Values are relative to the expression level of the non-infection group at each time (n = 4, *p < 0.05 compared to control group).](image-url)
Response of olIFNs gene expression in vitro was determined using DIT cell lines (derived from medaka hepatoma [21]). PolyI:C were transfected to DIT cell line to examine the response of olIFNs to intracellular polyI:C-stimulation. After 24 h transfection of polyI:C, expression levels of olIFNa genes were significantly higher than mock induction (Fig. 5A). Expression of the olIFNd gene was moderately increased after polyI:C transfection. Expression of the ISG15 gene was increased 12 h after polyI:C transfection and peaked at 24 h. However, expressions of GIG1a and ISG56 genes were not increased after polyI:C transfection. (It was noteworthy that expression of Mx genes was not detected in the DIT cell line). Responses of the 2 olIFN gene expressions to NNV-infection in the DIT cell line were also studied. Expression of olIFNa and olIFNd genes were significantly induced 12 h after infection, compared to the uninfected group (Fig. 5B). Furthermore, GIG1a gene expression was also significantly upregulated in response to NNV infection. Conversely, inductions of ISG15 and ISG56 genes were not detected after NNV-infection.

3.3. Biological activity of olIFNa and olIFNd

Biological activity of olIFNs in vitro was investigated. To generate products with optimal post-translational modifications (glycosylation and disulfide bond) and limit contamination by endotoxin, a mammalian cell line was used as the host to produce recombinant protein. The mature peptide sequence of olIFNa, olIFNd and EGFP (for negative control) cDNAs was cloned into the pFUSE-mIgG2a vector modified with the mouse IL-2 signal sequence and C-terminal histidine-tag (His-tag). The construct was transfected into a human embryonic kidney cell line (HEK293T cells). The His-tag-fused recombinant proteins were purified by 2 chromatographic columns (nickel-chelating sepharose and gel filtration). The molecular size of purified recombinant olIFNa and olIFNd (~26 kDa), as detected by western blot analysis, exceeded the predicted molecular size (Fig. 6A), perhaps because consensus sequences for the N-glycosylation site were based on amino acid sequences of olIFNa and olIFNd (Fig. 1B).
Biological activity of rolIFNa and rolIFNd proteins was assessed in vitro. Whole kidney cells of adult medaka were cultured with rolIFNa, rolIFNd and rEGFP as a negative control, or polyI:C as a positive control. After culture, total RNA was extracted, and expression level of ISGs determined by qRT-PCR (Fig. 6B). PolyI:C treatment significantly induced expression of the rolIFNa gene 1 h after treatment. Expression of the rolIFNd gene was moderately increased 12 h after polyI:C treatment and expression of GIG1a and ISG15 genes were also enhanced 3 h after polyI:C treatment. Furthermore, recombinant rolIFNa and rolIFNd induced expression of their own genes and expression of GIG1a and ISG15 genes slightly increased in rolIFNa-treated cells. In addition, rolIFNd also induced expression of the GIG1a gene. However, expression levels of Mx and ISG56 genes were not altered by any stimulator.

Biological activity of rolIFNs was investigated using DIT cells that were transfected, and qPCR was used to determine the expression level of ISGs. The fold change in expression was determined by normalization to the EGFP vector transfection control. Transfection of rolIFNa and rolIFNd successfully induced their own expression (Fig. 7A). Furthermore, expression of rolIFNa and rolIFNd genes were induced by overexpression of rolIFNd and rolIFNa, respectively. Transfection of rolIFNs induced gene expression of GIG1a and ISG15, but not ISG56. To determine antiviral effects of rolIFNs, DIT cells were treated with NNV 48 h after transfection with IFNa, IFNd or EGFP, viral RNA in the cells was quantified with qRT-PCR. In the EGFP group, viral RNA amounts increased from 24 h after NNV-infection (Fig. 7B). However, the amount of viral RNA was reduced in DIT cells transfected with rolIFNa and rolIFNd.

4. Discussion

Type I IFN genes have been reported in various teleosts and are regarded as the main regulator of antiviral responses. Medaka is a model fish widely used in biological research; adult fish and some cell lines are susceptible to red-spotted grouper and striped jack nervous necrosis viruses (NNV) [18,19]. However, identification of
type I IFN genes in medaka has apparently not been reported. In the present study, we identified 2 type I IFN genes in medaka. To date, type I IFN genes have been reported in diverse teleost species. The cysteine patterns of fish type I IFNs are divided into 2 major groups, namely 2C and 4C. In addition to phylogenetic analysis, teleost type I IFN genes are classified into 4 subgroups (i.e., a, b, c and d) [11]. Based on analysis of mature peptide sequence, both medaka type I IFNs belonged to the 2C group (Fig. S1). In addition, based on phylogenetic analysis, medaka type I IFN genes were classified into the subgroup-a and -d (Fig. 1A). It was previously suggested that superorder Acanthopterygii (pufferfish, flounder, and medaka) have only subgroup-d type I IFN gene. However, subgroup-a and -c type I IFN genes were recently identified in turbot (S. maximus) [17]. In this study, subgroup-a of type I IFN gene was identified. These findings may lead to identification of another subtype of type I IFN genes in superorder Acanthopterygii. Furthermore, perhaps subgroup-c and -b type I IFN genes exist in medaka as well in turbot. In the medaka Ensembl genome database, there were type I IFN receptors, CRFB5, CRFB1 and CRFB2. Although the number of type I IFN genes vary among teleost fish species, based on subgroups, type I IFN genes may be conserved in teleosts.

Both IFNs have potential glycosylation sites, 1 in olfIFNa and 2 in olfIFNd (Fig. 1B). Moreover, recombinant proteins of both olfIFNs had higher molecular weights than predicted molecular sizes (Fig. 6A). Almost all IFNs in teleosts have N-glycosylation sites. The glycosylated cytokines conferred the protein stabilization/solubilization effect of carbohydrate on structure, thereby extending half-life [22]. In that regard, N-glycosylation of teleost IFNs might promote antiviral activity.

Expression of teleost IFN genes has been reported to be induced by RNA viruses [6,15,23]. In the present study, there was enhanced olfIFNa and olfIFNd expression in vivo and in vitro by poly:C and in the kidney after NNV infection in vivo (Figs. 2–5). Furthermore, there were differences between olfIFNa and olfIFNd in their expression patterns after NNV-infection and poly:C stimulation. Similarly, differences in induction pattern among subtypes of type I IFNs have been reported in other species [6,15,23]. In salmon, induction of type I IFN expression differs among groups of IFN genes [6]. In that regard, it was suggested that salmon IFNa1/a2 genes are mainly induced through the RIG-I/MDA-5 pathway, whereas IFNb genes are mainly induced through the TLR7 pathway [6]. By contrast, in zebrafish, expression of 4C group IFN genes are predominantly regulated by RIG-I in NNV-infection [23]. It has been also reported that the expression patterns varies among tissues. In mammals, type I IFN produced in the lung of influenza virus-infected mice had STAT1-dependent induction of IRF7 and type I IFN gene expression [24]. However, the spleen constitutively expressed high levels of IRF7, and high expression was largely confined to plasmacytoid dendritic cells, which rapidly produce large quantities of multiple IFNα species after viral infection [24]. Therefore, it was concluded that the regulation of gene expressions of 2 olfIFN genes in response to an RNA-virus differed among tissues, cells, IFN groups, and species. Furthermore, contributions and responses of 2 IFNs genes to viral-infection could differ in medaka.

In renal hematopoietic cells derived from whole kidneys, rolIFNa and rolIFNd each induced expression of their own genes (Fig. 6B).
The rolIFNs-overexpressing DIT cells also had induction of 2 type 1 IFN genes (Fig. 7). Additionally, we demonstrated that rolIFNa moderately induced expression of GIG1a and ISG15 genes in renal hematopoietic cells (Fig. 6B). The rolIFNd protein also induced increased expression of GIG1a gene (Fig. 6B). In DIT cells, transfection of rolIFNa and rolIFNd genes induced expression of ISG15 and GIG1a genes and a reduction of viral titer after NNV-infection (Fig. 7). Gig1 [grass carp reovirus (GCRV)-induced gene 1] was firstly identified as ISGs from UV-inactivated GCRV-infected crucian carp (Carassius auratus) blastulae embryonic (CAB) cells [25]. In grass carp (Ctenopharyngodon idella), overexpression of GIG1 protects cells against virus infection [26]. Furthermore, ISG15 participates in antiviral defenses of mammals. There are also many reports regarding identification of ISG15 genes in various fish species [27–36]. It is clearly demonstrated that zebrafish ISG15 inhibits viral infection by RNA and DNA viruses in vitro [37]. Taken together, we inferred that medaka IFNs have anti-viral effects to induce expression of GIG1a and ISG15. Myxovirus resistance protein (Mx) is also a well-understood ISG in fish [8,38–40]. However, in this study, the Mx gene in renal hematopoietic cells was not induced by stimulation of either rolIFNs or polyI:C in vitro. The Mx genes have been cloned, with variations among species in the number of Mx isoforms. In channel catfish, 3 MX gene isoforms (Mx1-3) were identified [41]. Based on reporters assay, increased expression in response to polyI:C was only detected for Mx1. Although we could identify only 1 Mx gene in medaka fish, there may be another Mx gene produced in response to polyI:C and IFN stimulation in medaka [41]. In a turbot study, IFN1 (4C group) induced typical ISG expression including Mx, but not IFN2 (2C group) [17]. Therefore, perhaps expression of Mx gene in medaka fish is regulated by 4C group type I IFN in medaka.

In conclusion, we identified 2 types of type I IFN from medaka and classified each rolIFNa and rolIFNd based on phylogenetic tree analysis using mature peptide sequences. We also investigated in vitro biological activity of medaka type I IFNs. Expressions of both type 1 IFN genes were characterized after NNV administration...
in vivo and in vitro. Overexpression of oIFNs in DIT cells induced GIG1a and ISG15 gene expression and reduced viral titers following NNV infection. Therefore, we inferred that both oIFNa and oIFNd were produced in response to RNA-viral infection and that they upregulated antiviral activity via induction of ISGs. Perhaps these findings will stimulate additional comparative study of type I IFN genes in teleosts.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fsi.2015.11.036.

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