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Cloning and sequencing of rainbow trout (*Oncorhynchus mykiss*) interferon regulatory factor 7

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ABSTRACT

Interferon regulatory factor 7 (IRF7) gene was cloned from a subtractive cDNA library constructed with mRNAs obtained from rainbow trout (*Oncorhynchus mykiss*) macrophage cell line (RTS-11). Using expressed sequence tag clones of submitted IRF7 amino acid sequences, specific primers were designed. Results showed that IRF7 cDNA contains an ORF of 1251 nucleotides that translates into a 416 residues putative peptide. The 5' untranslated region containing 102 nucleotides and the 3' UTR of the transcript consists of 645 nucleotides. The start codon of this ORF is located at nt 103 to 105 and lies in favorable sequence context for the initiation of translation. Alignment between the rainbow trout IRF7 and others IRF7s indicated that the predicted trout IRF7 association domain was located in residues 204-371 aa. The rainbow trout IRF7 nucleotide sequence is most similar to fish IRF7 with 41% identity for crucian carp, *Carssius auratus*, 45% for zebrafish, *Danio rerio*, and to mammalian IRF7 with 23% for human, *Homo sapiens* and 7% for mouse, *Mus musculus* identity overall.

Keywords: Cloning, Sequencing, Interferon regulatory factor 7 (IRF7), cDNA, Rainbow trout

INTRODUCTION

Cells infected with a virus are stimulated to produce and secrete Interferons (IFN), which in turn induce a complex pattern of physiological changes, including the establishment of an antiviral state in as yet uninfected cells. Interferons are a family of multifunctional proteins involved in immune activation, regulation of cell growth and antiviral response. Interferons are divided into two groups depending on their molecular basis, type I IFNs (IFN-αs and IFN-β) are produced by a variety of cells upon virus infection, and type II IFN (IFN-γ) is produced by activated T cells and natural killer (NK) cells (Ohteki *et al* 1998, Richardson *et al* 2001). Interferons exert their functions by induction of several IFN-stimulated genes, including IFN regulatory factors (IRFs), a family of transcriptional regulators. To date, a number of these interferon-induced genes have been cloned and sequenced in fish, including Mx protein (Leong *et al* 1998). These discoveries can be attributed mainly to the enormous amount of information obtained from genome projects for the fugu and zebrafish, and the large increase of fish EST (expressed sequence tag) entries available in Gene bank (Richardson *et al* 2001). The major function of the IRF family includes the regulation of immune responses,

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especially those directed against viral infection. The interferon regulatory factors (IRFs) are a family of transcription factors which have been shown to play an essential role in the regulated expression of type I IFN genes, IFN-stimulated genes (ISG) and other cytokines and chemokines. The IRFs are a growing family of factors induced by IFN, cytokines, virus infection, cell stress or dsRNA. By now, nine family members have been identified in mammals, including IRF1 to 7, IRF8 (IFN consensus sequence-binding protein, ICSBP) and IRF9 (IFN stimulated gene factor 3γ/p48). Several IRFs homologues have also been cloned in avian, fish and amphibians (Leong *et al* 1998) but their exact function remains to be clearly characterized. In primative vertebrate a few orthologues of mammalian IRFs have been indentified. In fish, some members of the IRFs subfamily, including Japanese flounder *(Paralichthys olivaceus)* IRF2 (Yabu *et al* 1998, Marlowe *et al* 2005), pufferfish *(Fugu rubripes)* IRF (Richardson *et al* 2001), rainbow trout *(Oncorhynchus mykiss)* IRF1 and IRF2 (Collet *et al* 2003), zebrafish *(Danio rerio)* interferon gene (Chen *et al* 2005), and crucian carp *(Carassius auratus L.)* IRF7 (Zhang *et al* 2003) have been identified. This investigation presents the cloning and sequencing of rainbow trout IRF7 gene, which is an IFN regulatory factor that mediates signaling elicited by type 1 IFNs (IFNα and IFNβ). Rainbow trout is used as the study model, due to the economical value to the fish farming industry.

MATERIALS AND METHODS

Cell lines and induction. The rainbow trout RTS-11 macrophage cell line was used in this study as described previously (Yabu *et al* 1998; Richardson *et al* 2001). Cells were grown in culture medium also in suspension (Leibovitz L-15, Invitrogen) supplemented with 30% foetal calf serum, 100 IU/ml penicillin G and 100 µg/ml streptomycin (Invitrogen). Cells were maintained in 25 cm^2 flasks (Nunce) at -20 °C and passaged every 2 weeks. The cells were stimulated for 4 h with 20 µg/ml dsRNA polyinosinic: polycytidilic acid (poly I: C).

Total RNA extraction and cDNA synthesis. Total RNA was isolated from poly I: C-induced RTS-11 cells using the method described previously (Chomczynski & Saachi 1987). First strand cDNA synthesis was initiated at the poly (A) tail of mRNA using the Adaptor-oligo (dT) primer (Table 1). Five micrograms of total RNA and 0.5 µg Adapter-oligo (dT) (synthesized in Sigma Genosys) were mixed in a 12 µl volume, heated to 70°C for 10 min and chilled on ice (Collet *et al* 2003). Samples were adjusted with additional reagents in a final volume of 20 µl to the following conditions: 1×RT buffer (25 mM Tris-HCl, pH 8.3, 37.5 mM KCl and1.5 mM $MgCl_2$), 0.5 mM each dNTP, 200 U/µl reverse transcriptase (Bioscript). Reactions were incubated at 42 °C for 1 h, heat inactivated at 70 °C for 10 min and stored at -20 °C before PCR.The 5' UTR was amplified using oligo (dG) and R4 as the nested gene specific primer (GSP). The PCR products were purified using Qiagen PCR purification kit, and cloned into pGEM T-Easy vector (Promega). As for the 3' UTR, the same protocol was applied with primers Adaptor and F3 as the GSP1 for the first PCR, and then Adaptor and F4 as the GSP2 for the second PCR (Figure 1).

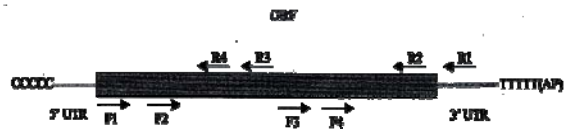

Figure 1. Map of the primers used to amplify the full sequence of the rainbow trout IRF7 gene.

PCR amplification and gel electrophoresis. By searching EST databases, several EST clones of IRF-7 amino acid sequences, were identified in carp (AY177629), zebrafish (BC065902), human

(AF076494), and mouse (NM016850). IRFs were aligned using ClustalW (Thompson *et al* 1994) and specific primers were designed from conserved regions using Primer 3 and Primer design tools on www.cybergene.se, to amplify IRF7 trout's unknowns regions (Table 1). PCR reaction contained: 1 X PCR buffer (16 mM $(NH_4)_2$ SO_4, 67mM Tris-HCl pH 8.8, 0.1% Tween-20), 1.5 mM $MgCl_2$, 50 µM each dNTPs, 0.4 µM each primer, 25 mU μl^{-1} Taq DNA polymerase (BioTaq, Bioline) and 1.5 µl cDNA mixed in a total volume of 25 µl. The cycling protocol was 1 cycle of 94 °C for 3 min, 32 cycles of 94 °C for 15 s, 56 °C for 15 s and 72 °C for 30 s, followed by 72 °C for 5 min. PCR products were separated on a 1.5% agarose-ethidium bromide gel. Three ml of purified PCR product (approx. 25ng) was ligated into pGEM T-Easy vector (Promega) in the following mix: 5 µl of ligation Buffer (30 mM Tris-HCl pH 7.8, 10 mM MgCl2, 10 mN DTT, 200 µM ATP, 5% polyethylene glycol), 1 µl of DNA ligase and 1 µl of pGEM T-Easy vector. Ten ml of ligation mix was added to 50 µl of TAM competent cells (ActiveMotif, Belgium) and plated onto McConkey Agar plates (Sigma) containing 100 µg ml^{-1} ampicillin. White colonies were chosen and checked for the presence of the insert by PCR. PCR-positive colonies were grown overnight in 4ml of LB medium containing 100µgml^{-1} ampicillin. Plasmid DNA was extracted and purified from 4ml of bacterial suspension following instructions of Qiagen's mini-prep kit. Two ml of purified DNA samples was checked with EcoR1 digestion at 37 °C for 90min and then sequenced by MWG-Biotech (Germany). After receiving the sequences from the 5' UTR, F1-R3, F3-R2, and 3' UTR, all the sequences were aligned on the EST known IRF7. In order to verify the IRF7 sequences of rainbow trout obtained during this experiments, a new pair of primers were designed (with software primer3 and primer design tools) to sequence the full length (Table 1).

Table 1. Oligonucleotides primers and adaptors sequences used in this study.

Name	Sequence 5' to 3'	Information
Forward (F)1	AAACCTCAGTT TGCGGACTGG	Designed against multiple alignment of known IRF7 sequences and used for PCR and RACE PCR
F2	TTCAGAGTCCC GTGGAAACAC	"
F3	TGCCACCAGTC CTAGTACAG	"
F4	AATGCTCTCGA GCCAAACAC	"
Reverse (R)1	GAGATACAGTA GTGCGATACC	"
R2	AGCTCCATCAG GCTGTTGTG	"
R3	GGTGTGTTTTC TGTAGGGATG	"
R4	TCTCTGTGCGG GCTCTCATAG	"
Adaptor oligo(dT)	AAGCAGTGGTA TCAACGCAGAG TACT(15)VN	Used for 3' RACE PCR
Adaptor AP	AAGCAGTGGTA TCAACGCAGAG	"
Oligo (dG)	GGGGGGGGGG GGG	Used for 5' RACE PCR
Full Length -F	TAGTACACGTC CACAGTCAAAC AGCCGACG	Used for Full length sequencing
Full Length -R	AGCTCCATCAG GCTGTTGTGGT GG	"

Sequence and statistical analysis. For sequence analysis, BLAST was used for the identification of homologous sequences in the GenBank databases (Altschul *et al* 1997). These sequences were aligned with the entire known IRF7 gene from other species, primers were designed from the conserved regions identified by alignment with carp, zebrafish, human and mouse IRF7 gene. Multiple alignments were generated using the CLUSTALW program (version 1.83).

RESULTS

Amplification results. Amplification of cDNA, from the RTS cells stimulated with the poly I.C with

primers IRF F and IRF R, gave various results: no amplification was obtained for F3-R1, F4-R2, F1-R1, F1-R2, F2-R1, F2-R2, F2-R3, and weak bands from primers F4-R1, F3-R2, and F2-R4. These were not used for cloning because they did not correspond to the expected size (F4-R1;F3-R2), or the amplification was too weak (F2-R4). The amplified products generated by F1-R3 or F1-R4, matched the expected size, and were ligated into pGEM T-Easy vector (promega) for cloning. PCR was repeated with F3-R1, R2 and F4-R1, R2 with a 58°C annealing temperature and amplified products obtained were >700bp for F3-R2 and >500bp for F4-R2. Products were ligated and cloned.

Cloning and sequencing of IRF7 5' and 3' UTRs. A strong band (around 500bp) appeared using 2 µl of the PCR product with oligo (dG) and R4 as the nested GAP2 to amplify the 5' UTR. The PCR products were purified using Qiagen PCR purification kit, and ligated into pGEM T-Easy vector (Promega) for cloning. For the 3' UTR, the same protocol was applied with primers Adaptor and F3 as the GSP1 for the first PCR, and then Adaptor and F4 as the GSP2 for the second PCR. Two strong bands were obtained around 800bp, and more than 1Kb. Compiled nucleotide and amino acid sequence for the trout IRF7 gene from the RTS cell line is shown in Figure 2. IRF7 cDNA contains an ORF of 1251 nucleotides that translates into a 416 residues putative peptide, with a 5' untranslated region (UTR) containing 102 nucleotides. The 3' UTR of the transcript consists of 645 nucleotides containing two mRNA instability motifs (ATTTA), with a polyadenylation signal (AATAAA) starting 23bp upstream from the poly A tail. The start codon of this ORF is located at nt 103 to 105 and lies in favorable sequence context for the initiation of translation with a purine Adenosine (A) in the -3 position and an other A in the +4 position. A computer homology search disclosed that the ORF contains highly conserved DNA-binding domain (DBD) at its amino-terminal region encompassing a characteristic repeat of tryptophan residues (Figure 3). In the DBDs, the identities reach 63% for carp, 59% for zebrafish, 46% for human and 43% for mouse. As the homology results with other submitted IRF7 were compared, rainbow trout IRF7 nucleotide sequence is most similar to fish IRF7 with 41% identity for crucian carp *Carssius auratus*, 45% identity for zebrafish *Danio rerio*, and to mammalian with 23% identity for human *Homo sapiens* and 7% identity for mouse *Mus musculus* overall.

DISCUSSION

In this study, the rainbow trout IRF7 cDNA was isolated, cloned and sequenced. Trout IRF cDNA isolated in this work appears homologous to fish and mammalian IRF7 based on the overall amino acid identity. Trout IRF7 comprised a main ORF of 1251 bp that translated to produce a putiative peptide of 416 amino acids. The carp and zebrafish IRF7 encode a similar length polypeptide of 421 and 423 aa. The rainbow trout sequence contains a DNA-binding domain, which is highly conserved among the IRF family; however, trout IRF7 has only four of five tryptophans repeats, due to the loss of the second tryptophan. In IRF1 structure, three of the five tryptophans (Trp11, Trp38 and Trp58) straddle the major groove containing the recognition helix and form hydrogen bonds contacting IFN stimulated response element (ISRE) and a mutation of the tryptophans is likely to affect both DNA binding and stability of the protein (Escalante *et al* 1998). In comparison to the human IRF1 structure, trout contains the tryptophans at a similar position to IRF1, meaning trout IRF7 could contact ISRE. Since fish lie at a low state of evolution, it is plausible that fish IRFs are less similar to their mammalian orthologues so that it is difficult to consider them orthologues of any specific mammalian IRF (Nehyba *et al* 2002).

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1       aaaacaactagaacacttacatagtagtacacgtccacagtagtacacgtccacagtagt

61      acacgtccacagtcaaacagccgacgcgtctaagcattaaagatgcaaagctgcaaacct
                                                  M  Q  S  C  K  P

121     cagtttgcggactggctgatagaacaggtgcggaccgagcaatacactggtttgttcttt      6
         Q  F  A  D  W  L  I  E  Q  V  R  T  E  Q  Y  T  G  L  F  F

181     attgacaacaataagttcagagtcccgtggaaacacaattctaggaaggattgctccgag     26
         I  D  N  N  K  F  R  V  P  W  K  H  N  S  R  K  D  C  S  E

241     gacgaccgtaaaatattcagggcatgggcagtggtcagtgggaaaatcaatgagcatccc     46
         D  D  R  K  I  F  R  A  W  A  V  V  S  G  K  I  N  E  H  P

301     actgacaaggcaaagtggaagaccaacttccgctctgcactgaacagcttgtgcaggcgt     66
         T  D  K  A  K  W  K  T  N  F  R  S  A  L  N  S  L  C  R  R

361     ttcaagatggtggaagaccactccaaggactcaaacgacccccataaagtctacctggtc     86
         F  K  M  V  E  D  H  S  K  D  S  N  D  P  H  K  V  Y  L  V

421     atcaatgagtataactatgagagcccgcacagagaagaaatcactctggagaactatggc    106
         I  N  E  Y  N  Y  E  S  P  H  R  E  E  I  T  L  E  N  Y  G

481     cttgactgtgctctcatccctacagaaaacacacccccaggcatggagcatgatatactg    126
         L  D  C  A  L  I  P  T  E  N  T  P  P  G  M  E  H  D  I  L

541     aactttagcaacctaaccttgaacccccctagacctgaaccaacacacagagaattctata    146
         N  F  S  N  L  T  L  N  P  L  D  L  N  Q  H  T  E  N  S  I

601     ccggtacacacacaccacccagtgccaccagtcctagtacagcagccatactaccaagta    166
         P  V  H  T  H  H  P  V  P  P  V  L  V  Q  Q  P  Y  Y  Q  V

661     aatccggatgccctgctcaacctgcctgccgcccactcatccctctgggatttggagatc    186
         N  P  D  A  L  L  N  L  P  A  A  H  S  S  L  W  D  L  E  I

721     accatctcctataggggggagcgagatgcgaaagacccaggtgtcaggccccagggttcag    206
         T  I  S  Y  R  G  S  E  M  R  K  T  Q  V  S  G  P  R  V  Q

781     ctccactaccagtgtaatgctctcgagccaaacacccagcccctctgcttccccagcact    226
         L  H  Y  Q  C  N  A  L  E  P  N  T  Q  P  L  C  F  P  S  T

841     gacgggttgccagaccacaaacagattgaatacaccaactgcatcctaggaagcgtccaa    246
         D  G  L  P  D  H  K  Q  I  E  Y  T  N  C  I  L  G  S  V  Q

901     aggggtcttctcctcgaggtacaaaacactggtatctatggttaccggcaggacaagtgc    266
         R  G  L  L  L  E  V  Q  N  T  G  I  Y  G  Y  R  Q  D  K  C

961     cacgtgttctccagtactagcgaccccagagaggcacaccctgaacccaggaagatgcct    286
         H  V  F  S  S  T  S  D  P  R  E  A  H  P  E  P  R  K  M  P

1021    caaaatgaaatggtacaactgttgagcttccagcagtacgaaaatgagctgatagcattt    306
         Q  N  E  M  V  Q  L  L  S  F  Q  Q  Y  E  N  E  L  I  A  F

1081    aaggagaacagacgaggctctcctgactataccatccacatgtgctttggtgagaagttc    326
         K  E  N  R  R  G  S  P  D  Y  T  I  H  M  C  F  G  E  K  F

1141    cctgacggaaaacccctggagaagaaactcatcattgtcaaggtggttcccctgatctgt    346
         P  D  G  K  P  L  E  K  K  L  I  I  V  K  V  V  P  L  I  C

1201    cgttacttccacgaggtggcccaggaggagggggcgtcgtccctccagaatgacatcagc    366
         R  Y  F  H  E  V  A  Q  E  E  G  A  S  S  L  Q  N  D  I  S

1261    ctgcagatctcccaccacaacagcctgatggagctcatcaatgccacctggcccgacggc    386
         L  Q  I  S  H  H  N  S  L  M  E  L  I  N  A  T  W  P  D  G

1321    ccacagcacgccatggggcagtacttctagaccaccaattgtaccacatcaccagccctt    406
         P  Q  H  A  M  G  Q  Y  F  *                                415

1381    atctagaactggtacctcactactgtatcacttaattgaatcttccaacaggaaagaaat

1441    acttagcaagtggatatgtaatgcaattgtgtacgactcaaacttgtagatctttgaaag

1501    tcagacaaatgttggtgttagatcagggctctccagcattgttccctgagagctacagtc

1561    ctataggttttcactcaaccctaatctagcacaccagacttgaataattagctggttgat

1621    aagccgactcaggttagttacaactggggttggagcgaaaacctacaggatggtagctct

1681    ccaggaacagggttggagagccctgtgttagattattctgatctatgatttgtgtcatgc

1741    agtagtataggctgtccttctcttgacgatgtgtgtatatatatttaaatatacatcaaa

1801    tcgctgtgtgttcgtaatgtgttcatgtatcatttagtggtatttttctcttccactgaat

1861    tcccttgtgtccctatgggccacactttaaacctgtctaggtcactcaacccttcagtct

1921    gttctctttttgtattgaaaaatctgttttattgtactgaaagtgagcaaactccacccctg

1981    gcattgaaaagcactttgtatagtattgcaaaacaagatcaataaactttgccttgtttc

1996    cctaaaaaaaaaaaa
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Figure 2. Compiled nucleotide and amino acid sequence for the rainbow trout IRF7 gene.The polyadenylation signal (aataaa) is in italics and to motif associated with mRNA instability (attta) are underlined.

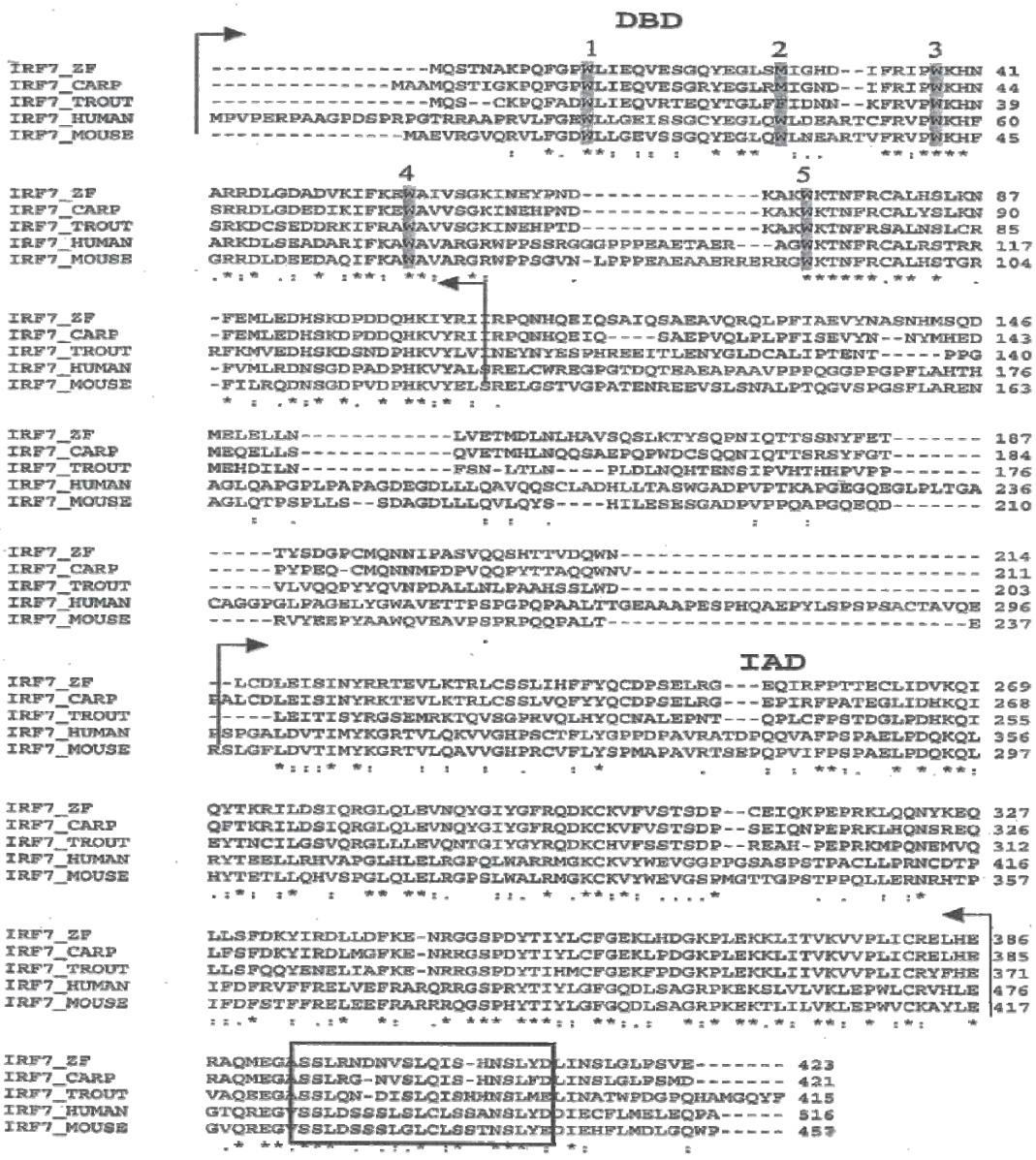

Figure 3. Multiple alignments of the rainbow trout IRF7 (Trout) with the IRF7 from zebrafish (ZF), carp, human and mouse. The putative DNA-binding domain (DBD) and interaction associated domain (IAD) are indicated by arrowed brackets. The conserved tryptophan (W) residues that constitute a "tryptophan cluster" are boxed in DBD and numbered. The predicted serine-rich domains are boxed in the carboxy-terminal region. Identical (*) and similar (. and :) residues identified by the Clustal W. program were indicated.

Alignment between rainbow trout IRF7 and others IRF7s indicated that the predicted trout IRF7 IRF association domain (IAD) was located in residues 204-371 amino acids.

Transactivation domain of Smad morphogens, termed the IAD, is involved in IRF protein -protein associations with other proteins and family members (Nehyba *et al* 2002).

It is well known that a conserved serine-rich domain has been characterized in the C-terminal region of mammalian IRF3 and IRF7 by the use of deletion mutants. The serine residues in domain are targeted for virus-induced phosphorylation that is required for the interaction between IRF3 and IRF7 or other IRF members. A C-terminal region homologue search showed that IRF7 also shared the serine- rich domain located in residues 378-397 aa. Structurally, all IRFs share significant homology in the amino-terminal (the first 115 amino acids), consisting of a highly conserved DBD characterized by a signature of five tryptophans or a "tryptophan cluster" Trp11, 26, 38, three of which contact DNA, recognizing similar DNA motif and ISRE (Escalante *et al* 1998). The rainbow trout sequence contains a DNA-binding domain characterized by tryptophan repeats. It appears that only four of five conserved tryptophan residues are present. In the DBDs, the identities reach 63% for carp, 59% for zebrafish, 46% for human and 43% for mouse. Further investigation should be performed in order to better characterize trout IRF7 gene, e.g by gene walking, to identify the promoter and the ISRE region. Multiple variants of IRF7 have been found and isolated in mammals, demonstrating that IRF7 plays a critical role in regulating expression of IFN and IFN-responsive genes to virus infection (Au *et al* 1998).

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J. and Miller W. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

Au, W.C., Moore, P.A., LaFleur, D.W., Tombal., B. and Pitha, P.M. (1998). Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon genes. *Journal of Biological Chemistry* 273: 2910-2917.

Chen, J.Y., You, Y.K., Chen, J.C., Huang, T.C. and Kuo, C.M. (2005). Organization and promoter analysis of the zebrafish (*Danio rerio*) interferon gene. *DNA and Cell Biology* 24: 641-650.

Chomczynski, P. and Saachi, N. (1987). Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate – Phenol - Chloroform Extraction. *Analytical Biochemistry* 162: 156-159.

Collet, B., Hovens, G.C.J., Mazzoni, D., Ikuo, H., Aoki, T. and Secombes, C.J. (2003). Cloning and expression analysis of rainbow trout *Oncorhynchus mykiss* interferon regulatory factor 1 and 2 (IRF-1 and IRF-2). *Developmental and Comprative Immunology* 27: 111-126.

Escalante, C.R., Yie, J., Thanos, D. and Aggarwal, A.K. (1998). Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. *Nature* 391: 103-105.

Leong, J.A.C., Trobridge, G.D., Kim, C.H.Y., Jonhson, M. and Simon. B. (1998). Interferon-inducible Mx proteins in fish. *Immunology Review* 166: 349-363.

Marlowe, C., Caipang, A., Hirono, I. and Aoki, T. (2005). Induction of antiviral state in fish cells by Japanese flounder, *Paralichthys olivaceus* interferon regulatory factor-1. *Fish & Shellfish Immunology* 19: 79-91.

Nehyba, J., Hrdlickova, R., Burnside, J., Henry, R. and Bose, H.R. (2002). A novel interferon regulatory factor (IRF), IRF-10, has a unique role in immune defense and is induced by the v-Rel oncoprotein. *Molecular and Cell Biology* 22: 3942-3957.

Ohteki, T., Yoshida, H., Matsuyama, T., Duncan, G.S., Mak, T.W. and Ohashi, P.S. (1998). The transcription factor interferon regulatory factor 1 (IRF-1) is important during the maturation of natural killer 1.1^+ T cell receptor-α/β^+ ($NK1^+$ T) cells, natural killer cells, and intestinal intraepithelial T cells. *The Journal of Experimental Medicine* 187: 967-972.

Richardson, M.P., Tay, B.H., Goh, B.Y., Venkatesh, B. and Brenner, S. (2001). Molecular cloning and genomic structure of a gene encoding interferon regulatory factor in the Pufferfish (*Fugu rubripes*). *Marine Biotechnology* 3: 145-151.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.

Yabu, T., Hirose, H., Hirono, I., Katagiri, T., Aoki, T. and Yamamoto, E. (1998). Molecular cloning of a novel interferon regulatory factor in Japanese flounder, *Paralichthys olivaceus*. *Molecular Marine Biology and Biotechnology* 7: 138-144.

Zhang, Y.B., Hu, C.Y., Zhang, J., Huang, G.P., Wei, L.H., Zhang, Q.Y. and Gui, J.F. (2003). Molecular cloning and characterization of crucian carp (*Carassius auratus L.*) interferon regulatory factor 7. *Fish & Shellfish Immunology* 15: 453-466.