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Short communication

Structural and expression studies of interferon regulatory factor 8 in Japanese flounder, *Paralichthys olivaceus*

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ABSTRACT

Interferon regulatory factor 8 (IRF8) plays a role in both innate and adaptive systems in mammals. In this study, the gene and promoter sequences of Japanese flounder, *Paralichthys olivaceus*, (Po) IRF8 were cloned, and its expression in response to polyinosinic:polycytidylic acid (poly I:C) and lymphocystis disease virus (LCDV) challenges was studied *in vivo*. The PoIRF8 gene spans over 3.3 kb with a structure of 9 exon–8 intron and encodes 420 amino acids. The putative protein shows the highest sequence identity (69.5–89.0%) to fish IRF8 and possesses a DNA-binding domain (DBD), an IRF-association domain (IAD) and a nuclear localization signal (NLS) of vertebrate IRF8. Phylogenetic analysis classified PoIRF8 into the cluster of fish IRF8 within vertebrate IRF8 group of IRF4 subfamily. A number of transcription factor binding sites were identified in the 2348-bp 5′ flanking region of PoIRF8 gene, including those of transcription factors for type I and type II interferon (IFN) inducible genes and genes regulating the development and function of lymphomyeloid cells in mammals. The PoIRF8 transcripts were expressed in all examined tissues of healthy flounders, with higher levels observed in the immune relevant tissues. They were up-regulated by both poly I:C and LCDV treatments in the spleen, head kidney, gills and muscle in an early phase of immune responses, with initiation and peak time points of induction prior to type I IFN and Mx. Relative to LCDV, the induction by poly I:C was quicker in all four tissues. These results indicate an involvement of PoIRF8 in the host's antiviral responses and a functional conservation of IRF8 between fish and mammals.

1. Introduction

Interferon regulatory factors (IRFs) with its 11 members were discovered as transcriptional factors principally regulating the transcription of interferon (IFN) and IFN-induced genes (ISGs) [1]. They play a role in virus-mediated signaling, early immune response to pathogens and hematopoietic cell development [2]. Structurally, all IRFs have extensive homology in the DNA-binding domain (DBD), which covers the first 115 amino acids where a cluster of 5 or 6 tryptophan residues is responsible for binding to target gene promoters [1]. The DBD binding sites are a class of similar DNA motifs with consensus sequences of N(G/A)AAAN$_{(1-2)}$(G/A/C)(A/G)AANN locating in the promoters of a diverse range of immune or immune-related genes. IRF3-10, possesses an IRF-association domain (IAD), whereas IRF1 and -2, and possibly IRF11, possess an IAD2, another type of association module of IRF proteins, both mediating formation of homo/heterodimers with other transcription factors [3]. Moreover, IRFs, except IRF6 and -10, possess one or two nuclear localization signals (NLS) related to nuclear translocation and reservation of IRFs at terminus regions [4]. Although the conserved binding domain in the family recognizes a similar DNA sequence, each IRF has distinct functional roles. IRF1, -3, -7 and -9 act constantly as transcriptional activators, while IRF2, -4, -5 and -8 are bi-functional factors that both activate and repress transcription depending on the target gene [5].

IRF8 in mammals, also termed interferon consensus sequence binding protein (ICSBP), was originally reported as a nuclear protein binding to interferon consensus sequence (ICS) in the major histocompatibility complex (MHC) class I gene promoter [6]. In contrast to IRF1 and -2, which are expressed in most cells, IRF8 is expressed predominantly in cells of immune system, such as

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myeloid and lymphoid cell lineages [7]. In mammals, IRF8 along with IRF4 acts as a critical growth determinant of hematopoietic cells. It is essential for the development of B and T cells as well as differentiation of myeloid progenitor cells [8]. It is also required for mature macrophage activity and production of type I IFNs both in dendritic cells (DCs) and plasmacytoid dendritic cells (pDCs) [9–11]. IRF8 usually interacts with Ets family member PU.1/Spi-1 which recognizes the Ets-IRF-E composite element (EICE) in target promoters to exert its transcriptional regulatory action. It has been demonstrated that IRF8 is induced preferentially by IFN-γ rather than by IFN-α/β and the induction by IFN-γ is rapid which doesn't depend on *de novo* protein synthesis, suggesting a nature of an immediate early gene for IRF8 in host's immune responses [6]. Further, IRF8 was reported as an important regulator of cross-talk between the toll-like receptor (TLR) and IFN-γ signaling pathways with respect to LPS-TLR4 and polyinosinic:polycytidylic acid (poly I:C)-TLR3 ligations [12]. More recently, IRF8 was found to synergize with IRF3 to regulate rapid IFN-β induction in human blood monocytes [13]. These findings, together with its function in the development of DCs subtypes and amplification of IFN production in pDCs, make IRF8 a likely target for poly I:C/virus-activated signaling pathways. As a bi-functional factor, IRF8 represses IRF1-mediated induction of MHC class I and IFN-β reporters in the absence of IFN treatment, DNA binding activity of IRF9, transcription of Fas-associated phosphatase 1, a protein-tyrosine phosphatase inhibiting Fas-induced apoptosis in myeloid cells, and TLR3 gene expression [14–17].

To date, fish IRF8 has been cloned and studied in rainbow trout, turbot and rock bream, and shown to be transcriptionally up-regulated by poly I:C, viruses, bacteria, IL-15, phorbol 12-

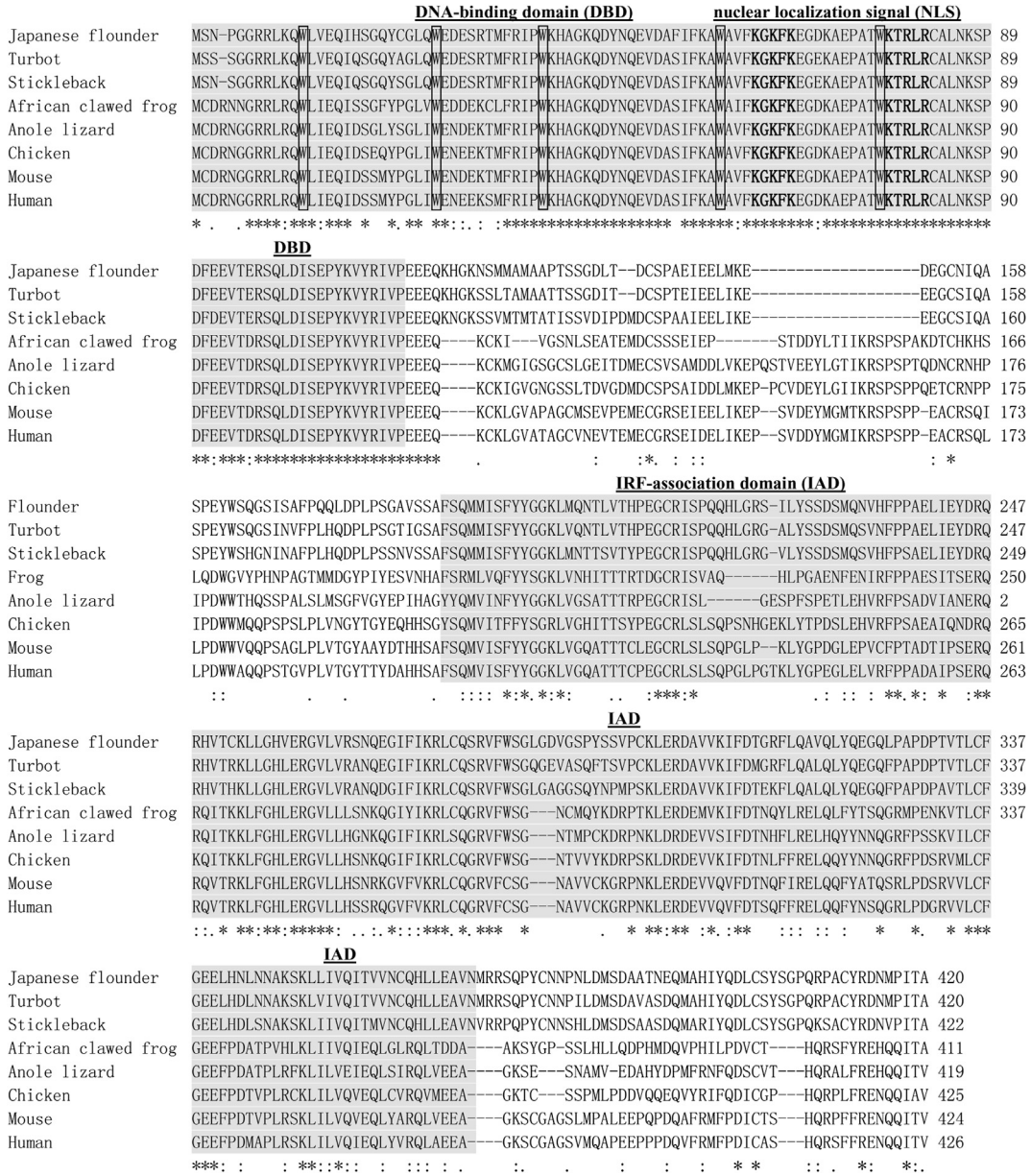

Fig. 1. Multiple alignment of PoIRF8 (Japanese flounder IRF8) amino acid sequence with other IRF8 proteins. The putative DNA-binding domain (DBD) and IRF-association domain (IAD) are shaded in gray. The bipartite nuclear localization signal (NLS, KGKFKn^{10}KTRLR) is bolded. The conserved tryptophan (W) residues composing a "tryptophan cluster" are boxed in the DBD. The residues identical in all sequences are shown with asterisks (*), whereas those with strong homologies and weak similarities are marked by colons (:) and dots (.), respectively. The accession numbers of the sequences are shown in Table 2.