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REVIEW ARTICLE

RIG-I-Like Receptors (RLRs) and Toll Like Receptors (TLRs) Mediated Regulation of Type I Interferons (IFNs) Signaling In Fish

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ABSTRACT
Inflammatory mediators known as cytokines (class II) which comprised of Interferons (IFNs) play vital roles in host immune defense and the discovery of IFNs is considered as a pioneer in the field of immunology. Fish type I IFNs may signal to the downstream receptors in a same way as in mammals. In zebrafish, two group IFNs such as IFN1/4 mediated signaling through CRFB1 and CRFB5 receptor complex whereas IFN2/3signal through CRFB2 and CRFB5 complex. Three families of pattern recognition receptors (PRRs) which includes retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), Toll-like receptors (TLRs), and cytosolic DNA sensors, are required in the type I IFN response in mammals.
Keywords: *Type I IFNs; RLRs; TLRs; JAK-STAT*

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INTRODUCTION

Discovery of Interferons (IFNs) play an essential role in the field of biomedicine especially in immunological studies for the past sixty years. Inflammatory mediators known as cytokines (class II) which comprised ofIFNs play vital roles in host immune defense[1], especially against viruses[2]. IFNs are categorised in to three types as type (I, II and III) based on their similarity in sequence, biological function and its organisation of genome[2]. Almost all nucleated cells respond to type I IFNs[3] [4]. This review attempts to summarize the recent discoveries on the type I IFNs systems in fish, which is regulated by RLRs and TLRs.

DISCOVERY AND CLASSIFICATION OF TYPE I IFNS

In 2003, three separate groups worked in zebrafish[5], green spotted pufferfish[6] and Atlantic salmon [7], identified first fish IFN gene. Many copies of type I IFN gene is present in fish similar to other vertebrate species based on their genome linkage, so the gene copy number varied from four in zebrafish to eleven in atlantic salmon[8] [9] and appear to exist in all fish species[10] [11]. Type I IFNs in fish was originally grouped into two types based on the number of cysteine residues (required indisulfide bond formation) as group I – contains two cysteine and group II – contains four cysteine residues[12]. Based on the order of discovery and location of chromosome, type I IFN gene copies are depicted by arabic numerals for instance, in zebrafish IFNs genes (four types IFNs 1-4) are classified into group I comprised of IFNs 1 and IFNs 4 whereas group II comprised of IFNs 2 and IFNs 3[5] [8] [13]. Complex type I IFNs with subsets a, b, c, d, e and f are distinguished in salmonids[14] [9]. IFNs subtype h is the newly added and identified in perciformes [15] [16] [17].

RECEPTORS OF TYPE I IFN MEDIATED SIGNALING PATHWAY

Type I IFN-mediated signalling pathway initiated with the interaction between type I IFNs and their receptors IFNAR1 and IFNAR2 (heterodimeric receptor complex) in mammals[1]. Researchers mainly immunologists suggested that the two receptors (IFNAR1 and IFNAR2) pertain to the class II cytokine

receptor family, which is known as cytokine receptor family B (CRFB) in fish[6] [18]. There are 17 CRFB members are present in zebrafish and pufferfish revealed in genome-wide sequencing. CRFB1 and CRFB2 showed homology to mammalian IFNAR2 whereas CRFB5 is homologue to mammalian IFNAR1[18]. In zebrafish, two group IFNs such as IFN1(IFNa) and IFN4 (IFNd) mediated signaling through CRFB1 and CRFB5 receptor complex whereas IFN2/3 (IFNc) signal through CRFB2 and CRFB5 complex[8] [19].

REGULATION OF TYPE I IFN-MEDIATED SIGNALING PATHWAY

In mammals, interaction between type I IFNs and their receptors stimulates the binding of tyrosine kinase 2 (TYK2) to IFNAR1 and Janus kinase 1 (JAK1) to IFNAR2 mediated through JAK-STAT pathway[20]. Phosphorylation of STAT1 and STAT2 (Signal Transducers and Activators of Transcription) by the aforementioned kinases are dimerized and put together with IFN regulatory factor (IRF) 9 to form an IFN stimulated gene factor 3 complex (ISGF3). Then the translocation of this trimolecular complex to the nucleus activate the transcription by binding to IFN-stimulated response elements (ISREs)[21](Figure 1). Copious immunogenetics studies revealed components of JAK-STAT pathway such as TYK2, JAK1, STAT1, STAT2 and IRF9, also exist in fish[22] [23][24].

Table 1. Conservation of JAK- STAT mechanism

Species	IFNAR1 IFNAR2	TYK1 TYK2	References
Mammals	Absence of enzymatic activity	Presence of Kinase activity	[25]
Fish	Absence of enzymatic activity	Presence of Kinase activity (Atlantic salmon TYK2)	[26]
Grass carp	Absence of enzymatic activity	Presence of Kinase activity (CRFB1 and CRFB5)	[27]

Interestingly, Fish type I IFNs may signal to the downstream receptors in a same way as in mammals. Two STAT1 genes (STAT1a and STAT1b) in zebrafish show similarity with human STAT genes. All five domains of human STAT1a is identified in zebrafish STAT1a at the same time, lack of C-terminal transcriptional activation domain is observed in both human and zebrafish STAT1b[28]. Zebrafish STAT1a is able to rescue IFN-mediated growth suppression in a STAT1-deficient human cell line, thus play an important role in type I IFN mediated signalling[29] [30].In congruent with this findings, phosphorylation and translocation of Atlantic salmon STAT1a in to nucleus was observed with recombinant IFNa1treatement[31]. In orange-spotted grouper, overexpression of STAT1a show antiviral activity against iridovirus and nodavirus by upregulating the ISGs expression[30].However, gibel carp STAT1(resembles like zebrafish and human STAT1b) induce ISG and inhibit viral infection[32]. Invitro studies in mandarin fish[16]and Co-IP assay in salmon[33]revealed that the ISGF3 complex (STAT1, STAT2 and IRF9) conserved in fish. At the same time, studies show that fish IRF9 is essential for the type I IFN-mediated signalling[34][35][36].

Numerous studies suggested that three families of pattern recognition receptors (PRRs) which includes retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), Toll-like receptors (TLRs), and cytosolic DNA sensors, are required in the type I IFN response in mammals[37]and in fish[38].

RLR-MEDIATED TYPE I IFN RESPONSE

Family of cytosolic receptors that is RLRs which recognize viral RNAs with three members, including RIG-I, melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2)[39]and the downstream molecules mitochondrial antiviral signalling protein (MAVS), and TANK binding kinase 1 (TBK1) are found conserved in fish.Upon the recognition of dsRNA from viruses, RLR components RIG-I/MDA5 recruits MAVS which then become associated with TRAF3 and TBK1, leading to the phosphorylation and activation of IRF3/IRF7 to elicit type I IFN response(Figure 1). RIG-I seems to be lost in fish of Acanthopterygii whereas MDA5 and LGP2 appear to exist in all fish species[40] [41].

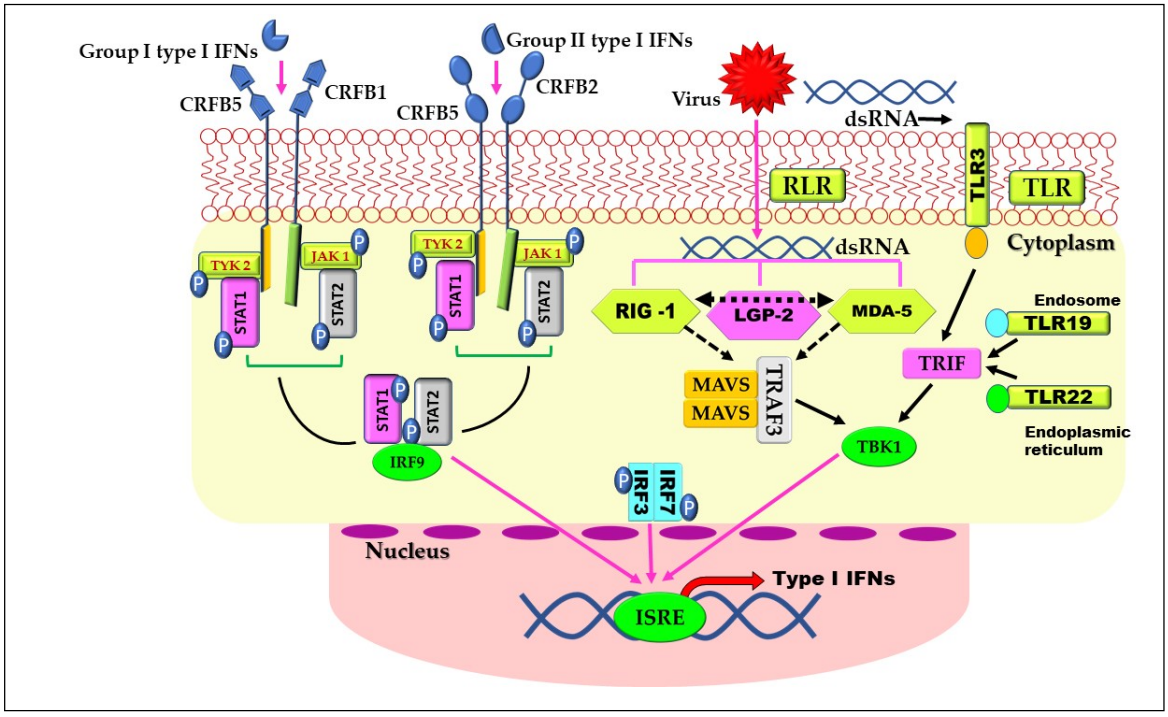
Notwithstanding, it remains to be determined whether there are multicopy genes STAT1 and STAT2 in a wide range of fish taxa. In addition, investigating the functional similarity and divergence of STAT1 and STAT2 fish multicopy genes in Type IFN signalling will be interesting.However, there are still two important and intriguing questions to be answered with regard to fish RLR-mediated type IFN response. First, since RIG-I appears to be lost in certain groups of fish species[40][41], clarifying the mechanism for

compensating for RIG-I deficiency in these species would be interesting. Second, much more research is needed to fully elucidate the dual roles of Type I IFN-mediated antiviral response fish LGP2 and to understand the factors that influence the functional switch of LGP2 in fish[42].

TLR-MEDIATED TYPE I IFN RESPONSE

Type I integral proteins also known as TLRs comprising an ectodomain containing ligand-rich repeats (LRRs), a transmembrane region and cytosolic Toll-IL-1 receptor (TIR) domains that mediate downstream signaling pathways[43]. TLR3, TLR19 and TLR22 are mainly involved in the activation of type I IFN response in fish[44] [45]which comprises a larger TLR collection, which shows similarity to mammalian TLRs and non-mammalian TLRs [46]. Among the three TLRs in fish, TLR3 is localized in endoplasmic reticulum and recognizes short dsRNA, TLR22 is located particularly in plasma membrane and recognizes long dsRNA and recruit TRIF to elicit type I IFN response [47] [48](Figure 1). The studies from *Takifugurubripes* commonly called fugu/puffer showed the ligand recognition and type I IFN-inducing activity of fish TLR3 and TLR22 whereas TLR19 response identified in grass carp.

In other fish species, the functional properties of these TLRs remain to be further characterized. Furthermore, although fish TLR9 may bind CpG-containing DNA as in mammals [49][50], it remains to be shown whether it can activate Type I IFN production. Future studies are also required to determine whether ssRNA can be conservatively recognized by fish TLR7 and TLR8 and to trigger type IFN response[42].



igure 1: Signalling pathway model for type I IFNs in fish. Two groups of IFNs interact with its common receptor CRFB5 and the two different receptors CRFB1 and CRFB2. Upon ligand – receptor interaction TYK2 and JAK1 are recruited and activated, leads to the phosphorylation of STAT1 and STAT2 which become dimerized and form a trimolecular complex with ISGF3, ultimately translocates to the nucleus and binds to ISREs, thus activating the transcription. Upon the recognition of dsRNA from viruses, RLR components RIG-I/MDA5 recruits MAVS which then become associated with TRAF3 and TBK1, whereas TLR3, TLR19 and TLR22 which recruit TRIF and TBK1 leading to the phosphorylation and activation of IRF3/IRF7to elicit type I IFN response.

CONCLUSIONS AND FUTURE PERSPECTIVE

First, since RIG-I appears to be lost in certain groups of fish species, it would be interesting to clarify the mechanism in these species to compensate for the RIG-I deficiency. Second, much more research is needed to fully elucidate the dual roles of Type I IFN-mediated antiviral response of fish LGP2 and the factors influencing the functional switch of LGP2 in fish. Future studies are also needed to determine whether fish TLR7 and TLR8 can conservatively recognize ssRNA and trigger the response of type I IFN.

CONFLICTS OF INTEREST

All authors disclose that there are no conflicts of interest that could inappropriately influence the outcome of the study.

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