DOES INTERFERON (IFN)S EXIST IN CRUSTACEA?

Dewi Syahidah*)# and Leigh Owens**)

*) *Research Institute for Mariculture, Gondol, Bali*
**) *James Cook University (JCU) Australia*

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ABSTRACT

The wide exploration of interferon (IFN)s in vertebrates for medical purposes has attracted researchers to investigate the existence of a similar role of interferon in other organisms such as invertebrates, including insects, and crustacea. A review of the literature indicates that there is no evidence of interferon existing either in insects such as *D. melanogaster* and *A. gambiae* which have had their genomes fully sequenced or in crustacea. However, a nonspecific antiviral state in crustacean, such as *P. monodon* can be efficiently triggered by both dsRNA and siRNA. The evidence suggests that anonymous cytokines, similar to interferon and not identical to any vertebrate IFNs, related to antiviral protection, do exist in crustacea. However, how widely spread of interferon immune response inducer or interferon-like molecules in this group is an important issue that remains to be explored.

KEYWORDS: interferon, crustacea, tiger shrimp

INTRODUCTION

Cytokines and interleukins (ILs) are soluble mediators that regulate the effectors' phase of immune responses (Ottoviani *et al*., 1995). One multigene family of the cytokines, interferon (IFNs) was identified in the late 1950s as the vertebrates' major innate antiviral response against several viral infections (Samuel, 2001). Interferon work by inducing viral and tumour resistance, modulate the effect of the immune-system, and regulate the differentiation of cells (Gutterman, 1994; Guidotti & Chisari, 2001; Pang *et al*., 2005). This finding precipitated several studies to explore the antiviral function of interferon. In fact, the use of IFNs for medical purposes, particularly for humans has developed since 1970s when interferon was successfully analysed and characterised, leading to the successful production of type I (IFN-α and IFN-β) for chemical, biological, and immunological studies (Pestka, 2007). Soon after this success, several assays such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) and their kits have been widely developed to measure interferon (Fairchild & Moorhead, 1985; Le Moal *et al*., 1989; Paasch *et al*., 1996; Siebert & Larrick, 1992; Lessard *et al*., 1998).

The Food and Drug Administration (FDA), USA has approved both interferon type I and type II to be used for medical therapies. For instance, IFN-α is accepted for treatment of viral hepatitis, IFN-β is permitted in sclerosis therapy, and IFN-γ is approved in the chronic hereditary immune disorder, chronic granumalotous disease (Samuel, 2001). The approval paved the way for the commercial production of the drugs. In fact, the worldwide annual sales of the prescribed IFN-cytokine products recently valued well over US$1 billion

Corresponding author. Research Institute for Mariculture, Jl. Br. Gondol, Kec. Gerokgak, Kab. Buleleng, Kotak Pos 140, Singaraja, Bali 81101, Indonesia. Tel.: +62 362 92278
E-mail address: dewi_rimg@yahoo.com

(Kunzi & Pitha, 2005). Considering the important role of IFNs for therapeutics and their values, it is therefore highly significant to discover the functions of the IFN-stimulatory gene products in the hope of categorizing extra pathways that will facilitate our understanding of the important biological events influenced by IFNs or IFN-like molecules in various organisms.

This literature review will describe the works of interferon or interferon-like molecules in both mammal and non-mammal vertebrates, including humans and bony fish and in invertebrates, including insects and crustacea based on past literatures. Understanding how these fundamental processes work in anti-viral immunity may facilitate how to manage pathogens of both species. In addition, the knowledge of interferon provides a great hope for addressing disease problems in crustacean culture.

VERTEBRATES' INTERFERON RESPONSES AGAINST VIRAL INFECTION

In Humans

The study of human (*Homo sapiens*) immunity demonstrated that in response to viruses, cytokine signals regulation of cell growth and development of hematopoietic tissues are triggered by interferon regulatory factors (IRFs) (Paun & Pitha, 2007). These regulators play important roles within infected cells, facilitating viral, bacterial, and interferon-induced signalling pathways (Samuel, 2001). Previous studies confirmed that there are virus encoded analogous of cellular IRF and nine recognised human IRF genes, including *IRF-1*, *IRF-2*, *IRF-3*, *IRF-4*, *IRF-5*, *IRF-6*, *IRF-7*, *IRF-8*, and recently, *IRF-9* (Nguyen *et al*., 1997; Barnes *et al*., 2002). The understanding of the molecular mechanism of the pathogen induced innate antiviral response was significantly enhanced by the identification of two IRF, namely, IRF-3, and IRF-7 and their role in the transcriptional activation of type I *IFN* genes (Ronco *et al*., 1998; Au *et al*., 1998; Marie *et al*., 1998).

The fast production of interferon is the basis of the innate antiviral responses (Erickson & Gale, 2008). For these purposes, the activation of IRF-3 and IRF-7 which is crucial to the expression of IFN is suggested to be stimulated by distinct cellular receptors and signalling pathways (Akira *et al*., 2006; Honda *et al*., 2005). These two IRFs are triggered as downstream effectors of pathogen recognition receptor (PRR) signalling cascades (Erickson & Gale, 2008). PRRs are cellular proteins of the RNA-helicase family such as retinoic acid inducible gene-I (*RIG-I*), melanoma differentiation-associated protein-5 (MDA5), and the family of Toll-like receptors (TLRs) that employ particular viral products and signal to the nucleus to activate the expression of interferon-messenger RNA (IFN-mRNA) (Saito & Gale, 2006).

IRF-3, which is constitutively present in the majority of cells provokes a preliminary signal for the expression of IFN from the infected cells that is enlarged upon successive activation and expression of *IRF-7*, which is also an interferon-stimulated gene (*ISG*) (Tamura *et al*., 2007). It is important to note that the expression of interferon by the main interferon producers, plasma cystoids dendritic cells (pDCs) is mainly determined by IRF-7 as the key transcription factor (Honda *et al*., 2005). It is totally responsible for the induction of IFN antiviral defences (Honda *et al*., 2005). The character of *IRF-7* as an *ISG* demonstrates an essential predicament in immunity in which high intensity of IFN production and antiviral defences depends on the rate-limiting transcription of nuclear signalling of *IRF-7* mRNA and ensuring the production of protein (Erickson & Gale, 2008).

The synthesis of interferon is triggered by the induction of a cell corresponding to either IFN-α and -β (viral infection) or IFN-γ (antigen or mitogen stimulation). The replication of the virus is blocked in the IFN-treated cell in which synthesis of IFN-regulated proteins is induced by the action of paracrine-IFN (Figure 1).

Studies using biochemical, genetic, and cell-based approaches indicated that the cellular repressor proteins of translation initiation, eukaryotic translation initiation factor-4E (eiF4E)-binding proteins (4E-BPs), namely 4E-BP1 and 4E-BP2 played a key role in the synthesis of protein by stimulating mRNA translation as well as acting in the control of cell proliferation (Lawrence & Abraham, 1997). 4E-BPs are also key players in regulating the production of type-I IFN through translational contr-ol of *IRF-7* expression (Figure 2A) that confers rapid protein production from pre-existing but conversional silent mRNA pools (Colina *et al*., 2008).

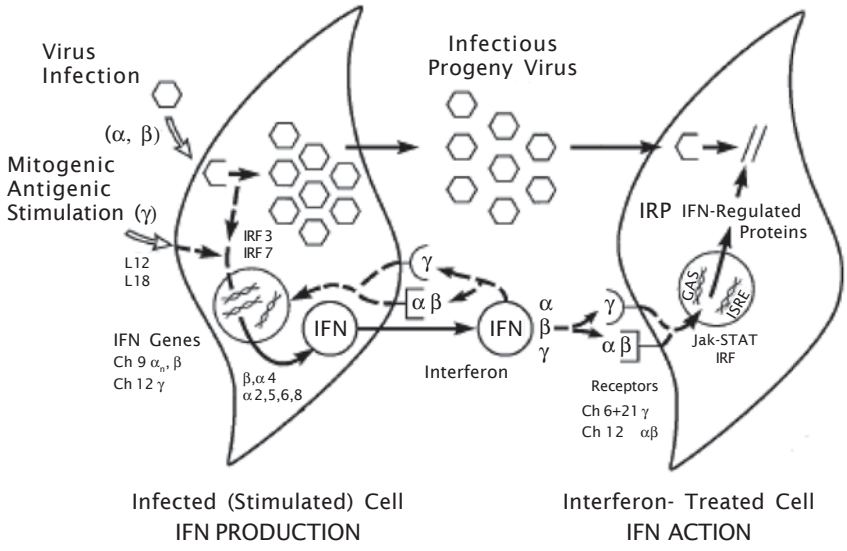

Figure 1. Production (left) and action (right) of IFN (modified from Samuel, 2001)

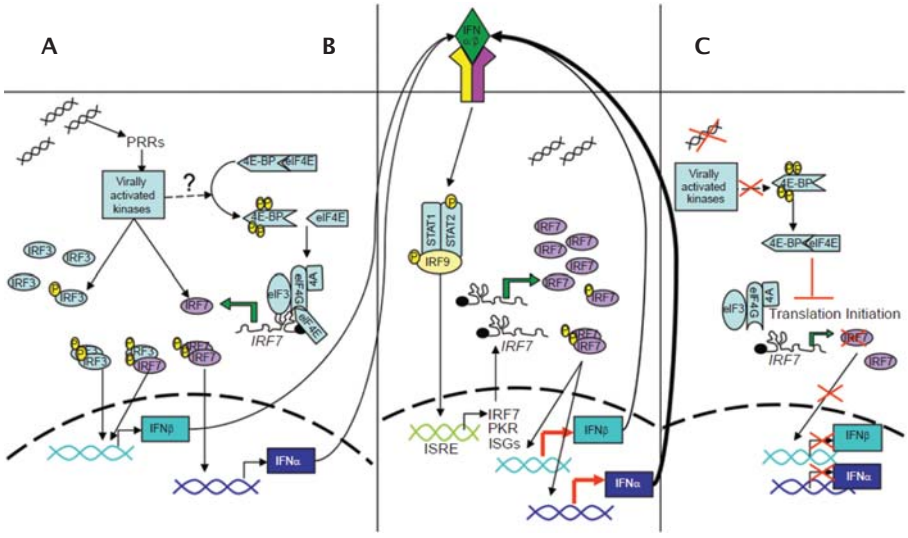

Figure 2. Steps in the response of interferon through 4E-BP translational suppression of *IRF7*. A. Activation of distinct signalling cascades; B. Activation of JAK/STAT pathway; C. Turning off the innate immune response by decreasing the production of interferon (fully explanation can be viewed in Erickson & Gale, 2001)

The interferon response begins upon virus infection, allowing PRRs to bind to their respective viral ligands, resulting in the activation of distinct signalling cascades. The mammalian target of rapamycin (mTOR) or an undefined protein kinase may lead to hyperphosphorylation of 4E-BP, allowing release of the eIF4E, which is now free to bind to eIF4G, forming the eIF4F translation initiation complex (Figure 2A). This increase in the pool of functional eIF4F allows the translation of mRNAs, such as IRF-7 mRNA, that were only

inefficiently translated or translationally silent under conditions of 4E-BP hypophosphorylation. PRR signalling triggers the phosphorylation of IRF-7 and IRF-3 by virus-activated kinases to induce their activation and translocation to the nucleus, resulting in transcriptional activation of type I *IFN* genes. In the next stage, the secreted IFNs bind to their cognate receptor, interferon associated receptor-1/-2 (IFNAR1/2), activating the Janus kinase (JAK) signal transducer and activator of transcription (STAT), the JAK/STAT pathway wherein the transcription factor, Interferon stimulated gene factor-3 (*ISGF-3*) which induces the expression of *ISGs* including *IRF-7*, *RIG-I* and RNA-dependent protein kinase (PKR*)*, and many others (Figure 2B). ISG products limit viral infection and enhance host immunity. After the viral clearance from the cells, it is imperative that the IFN response be dampened before it is harmful to the host. This may occur in part through translational-suppressive actions of the 4E-BPs on IRF-7 mRNA translation, which in turn decreases IFN production and turns off the innate immune response (Figure 2C).

Interferon in Bony Fish (Teleosts)

A study on innate immunity of fish demonstrated that TLRs recognise the pathogen-associated molecular patterns (PAMPs) of microorganisms that can trigger the expression of inflammatory cytokines and genes with antimicrobial activity through the activation of nuclear factor kappa B (NFκB) (Ewart & Tsoi, 2004). So far, a number of *TLR* genes and copy-DNA (cDNA)s have been identified in several fishes. Interestingly, the genome sequence of the pufferfish revealed clear similarity (orthologs) of mammalian *TLR 2*, *3*, *4*, *5*, *7*, *8*, and *9*, a proof that the genes have evolved from a common ancestor. In fact, the fish genome also encoded a *TLR* with an equally high sequence identity to mammalian *TLR 1*, *6*, and *10*. In general, the Toll signalling pathway runs through several phases (Figure 3). Initially, the exogenous ligands stimulate TLR to recruit two adaptor molecules, myeloid differentiation-88 (MyD88) and tumor necrosis factor (TNF) receptor associated family-6 (TRAF6). As a consequence, two kinases, namely mitogen-activated protein (MAP) kinase or threonine-activated kinase (TAK-1) and Iκkinase (IKK) are activated through subsequent phosphorylation. The next stage, in example, the inactivation of IκB by phosphorylation, releasing the nuclear transcriptional factor kappa-B (NFκB) into the nucleus by which the transcription of immune and inflammatory genes is activated (Ewart & Tsoi, 2004).

Several homologs of interferon in several bony fish has been cloned and characterised over the last decade. For instance, in zebra fish (*Danio rerio*) (Igawa *et al*., 2006); Japanese pufferfish (*Tetraodon nigroviridis*) (Zou *et al*., 2004), trout (*Oncorhynchus mykiss*) (Zou *et al*., 2005), Atlantic salmon (*Salmo salar*) (Robertson *et al*., 2007), catfish (*Clarias gariepenus)* (Milev-Milovanovic *et al*., 2006), common carp (*Cyprinus carprio*, L.) (Stolte *et al*., 2008) and goldfish (*Carassius auratus*) (Grayfer & Belosevic, 2009). Two isoforms of IFN-γ , which differ markedly within each species in sequence homology and expression patterns apparently exist in zebrafish (*D. rario*), catfish (*C. gariepenus*), carp (*C. carprio*, L), and goldfish (*C. auratus)* (Igawa *et al*., 2006; Milev-Milovanovic *et al*., 2006; Stolte *et al*., 2008). Various IFN and IFN-like activities in fish have been recently identified (Table 1.)

Interferon stimulatory genes *(ISGs)* were identified in rainbow trout (*O. mykiss*), namely *Mx1* and *Vig-2* whereas *ISG15* was identified in goldfish (*C. auratus*) and Atlantic cod (*Gadus morhua*) (Collet & Secombes, 2001; Boudinot *et al*., 2001; Liu *et al*., 2002; Seppola *et al*.,

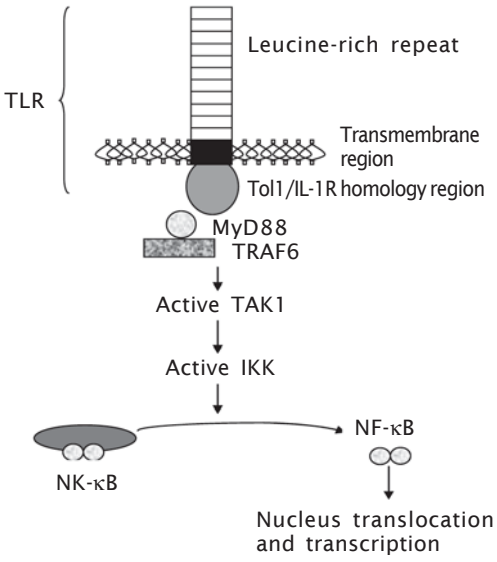

Figure 3. Toll-signalling pathways in teleosts (Ewart & Tsoi, 2004)

Table 1. IFN and IFN-like activities identified in fish (adopted from Schultz *et al*., 2008)

(MAF: Macrophage Activating Factor)

2006). Among the *IRFs* present in teleosts, *IRF-1* has been genetically copied and differentiated from the Japanese flounder (*Paralichthys olivaceus*), pufferfish (*T. nigroviridis*), rainbow trout (*O. mykiss*), turbot (*Scophthamus maximus*), and sea bream (*Sparus aurata*) (Yabu *et al*., 1998; Richardson *et al*., 2001; Collet *et al*., 2003a,b; Ord´as *et al*., 2006). *IRF-2* has been described in rainbow trout (*O. mykiss*) and mandarin fish (*Siniperca chuatsi*), and is homologous to the recognized vertebrate *IRF-2* (Collet *et al*., 2003a, b; Sun *et al*., 2006). In addition, *IRF-7* has been cloned from goldfish (*C. auratus*) and mandarin fish (*S. chuatsi)* (Zhang *et al*., 2003; Sun *et al*., 2007). These studies also demonstrated that the IRFs identified in fish possess substantial attributes of mammalian *IRFs*.

There are three major factors, including virus, interferon, and a synthetic double stranded RNA which is a polyinosinic: polycytidylic acid (poly I:C) are experimentally used to trigger *IRF* genes in fish (Collet *et al*., 2003, Zhang *et al*., 2003; Ord´as *et al*., 2006; Sun *et al*., 2006; 2007). For instance, *IRF-1* in flounder (*P. olivaceus*) was triggered by poly I:C (Collet *et al*., 2003a, b; Ord´as *et al*., 2006). *IRF-2* was triggered by poly I:C in trout (*O. mykiss*), and mandarin fish (*S. chuatsi*) (Collet *et al*., 2003a, b; Sun *et al*., 2006). Another gene, *IRF-7* was up-regulated by virus-infection or treatment-based IFN in goldfish (*C. auratus*), and induced by poly I:C in mandarin fish (*S. chuatsi*) (Zhang *et al*., 2003; Sun *et al*., 2007).

The induction of poly I:C in snakehead (*Channa argus*) raises the expression of a cDNA encoding *Carassius auratus* IRF genes (*Ca-IRF)s* in the spleen and liver (Jia & Guo, 2008). It was confirmed by real time PCR (RT-PCR) analyses that *CaIRF-1* and *CaIRF-2* genes for *IRF-1* in flounder (*P. olivaceous*), pufferfish (*T. nigroviridis*), trout (*O. mykiss*), turbot (*S. maximus*), and sea bream (*S. aurata*) (Yabu *et al*., 1998; Richardson *et al*., 2001; Collet *et al*., 2003a, b; Ord´as *et al*., 2006) and for *IRF-2* in trout (*O. mykiss*), and mandarin fish (*S. chuatsi*) (Collet *et al*., 2003a, b; Sun *et al*., 2006) were constitutive expression in a wide range of living-isolated tissues. However, *CaIRF-7* is primarily in the intestine, gill and peripheral blood, and varies from goldfish (*C. auratus*) *IRF-7* gene in a number of tissues (Zhang *et al*., 2003). Remarkably, significant levels of expression of three *CaIRF* genes were highly identified in the intestine and gill (Zang *et al*., 2003). This possibly an indication of the reality that they are the entrance organs through which antigens including microbes invade the fish body, and where the immune response of the fish is instigated (Jia & Guo, 2008). To date, a number of vertebrate *IRFs* genes and their protein sequences are available in GenBank (Table 2).

INVERTEBRATES' IMMUNE RESPONSES AGAINST PATHOGENS

On the contrary with vertebrates, there is still a lack of information at cellular or molecular level regarding invertebrates' immune response against viral infection because there is an absence of genes homologous to IFNs or to the main effectors of the IFN responses

Table 2. Various vertebrate *IRF* genes which are deposited in GenBank (Modified from Jia & Guo, 2008)

Organisms	Common gene names	Accession number	Organisms	Common gene names	Accession number
Human (*H. sapiens*)	IRF-1	NM_002198	Pufferfish (*T. nigroviridis*)	IRF-7	CAG02387
	IRF-2	NM_002199			
	IRF-3	Q14653			
	IRF-4	U52682			
	IRF-5	Q13568			
	IRF-6	AF027292			
	IRF-7	NM_001572			
	IRF-8	NP_002154			
	IRF-9	Q00978			
Trout (*O. mykiss*)	IRF-1	AF332147	Flounder (*P. olivaceus*)	ARF-1	AB005883
	IRF-2	AY034055			
Zebrafish (*D. rerio*)	IRF-1	AAH85555	Snakehead (*C. argus*)	IRF-2	EF067849
	IRF-6	AAX57954		IRF-2	EF067850
	IRF-7	AAH58298		IRF-7	EF067848
Goldfish (*C. auratus*)	IRF-7	AY177629	Seabream (*S. auratus*)	IRF-1	AAY68281
Takifugu (*T. rubipes*)	IRF-1	AF242474	Mandarin fish (*S. chuatsi*)	IRF-1	AAV65042
Turbot (*S. maximus*)	IRF-1	AAY68278			

(Adams *et al*., 2000; Meurs *et al*., 1990). A more detailed approach to study the invertebrate immune system is required.

In Insects

Drosophila melanogaster has been widely used in the study of the invertebrate immune system as it has genetic malleability, a lack of a conventional mammalian adaptive immune system and the conservation of these signalling pathways with those of higher organisms. In addition *Drosophila* possesses a robust RNA interference (RNAi) system homologous to the post-transcriptional gene silencing (PTGS)/ RNAi systems similar to that in plants and other animals (Zambon *et al*., 2006).

As the genome of this fruit fly species has been completely sequenced (Adams *et al*., 2000), it has been widely accepted that invertebrates such as *Drosophila* lack of double-stranded RNA (dsRNA)-induced immune responses as a consequence of the absence of genes identical to IFNs or to the main effectors of the IFN such as RNA-dependent protein kinase (PKR) response (Robalino *et al*., 2004). However, the discovery of dsRNA-mediated posttranscriptional gene silencing or (PTGS) or RNA interference (RNAi) in *Drosophila* confirmed the complex properties of dsRNA in isolated living cells (Misquitta *et al*., 1999).

The discovery begins when an identical phenotype was observed with the removal of the *nautilus* gene product by genetic interference upon experimental injection of *nautilus* dsRNA (Misquitta *et al*., 1999). It also defined a crucial role for *nautilus* (*nau*) which is a single family member of Myogenic regulatory factor (MRFs) in *Drosophila* during embryonic muscle formation, providing a powerful approach of RNAi for the targeted disruption of a given genetic function in *Drosophila* (Misquitta *et al*., 1999).

In the further observation application of RNAi that was suggested correlated with an

early antiviral mechanism involved in the limitation of viral gene product expression in infected cells as a result of the occurrence of viral dsRNAs during the course of infection as controls created phenotypes that are essentially indistinguishable from the original mutation (Misquitta *et al*., 1999).

Interestingly, the specific Janus kinase/ signal tranducer and activator of transcription (JAK/STAT) pathway and STAT-like molecules which are crucial in response to the stimulation of cytokine persist throughout animal evolution. Roughly, two STATs have been detected in the nematode, *Caenorhabditis elegans* (Liu *et al*., 1999), and three STAT-like molecules have been identified in the slime mould, *Dictyostelium* even though to date, no invertebrates have been identified to posse JAK-like protein kinases (Zeidler *et al*., 2000). What is more interesting to note in the immune systems of other invertebrates such as *Drosophila melanogaster* and *Anopheles gambiae* is that there is the evidence of the JAK/STAT as the signal transduction pathway identical to those well-known to control vertebrate IFN response (Zeidler *et al*., 2000; Christophides *et al*., 2002). In *Drosophila*, for example, the JAK/STAT pathway is not only involved in the control of haemopoiesis and sex differentiation, the segmentation of the embryo and the establishment of polarity within the adult compound eye but also partially worked in the expressed an immune role (Zeidler *et al*., 2000; Zambon *et al*., 2005). The identification of 242 *A. gambiae* genes from 18 gene families implicated in innate immunity have demonstrated marked diversification virtual to that of *Drosophila melanogaster* (Christophides *et al*., 2002). The result confirmed that *Anopheles* has four Toll genes, namely *Toll 6*, *7*, *8*, and *9* that are clear orthologs of *Drosophila* counterparts. It also confirmed that other single genes such as *My88*, *Tube*, *Pelle*, and *Cactus* that were recognized in *Anopheles* were also ortholog with those in *Drosophila* and possess important role in the Toll signal transduction pathway (Christophides *et al*., 2002).

Crustacean Immune Responses

The complicated constraints in crustacean immune systems have led to several ineffective therapeutants such as immunostimulant and vaccines being used for rearing shrimps (Smith *et al*., 2003). These practices should be slowly phased out because it is believed they can trigger detrimental long term effects, including the risk of contamination to the environment and the final food product (Grant & Briggs, 1998). They also contribute to the spread of drug resistant pathogens (Smith *et al*., 1994). Another constraint is a basic theory that the crustacea lack specific immunity such as that initiated in vertebrates and that they depend on pattern recognition protein (PRP) to counteract pathogens (Flegel, 2007). However, this theory should not exclude specific antiviral activities involved in crustacean which is until recently, still questionable. Several attempts have been made to figure out the role of antiviral responses in crustaceans.

It has been believed that microbial products such as lipopolysaccahrides or lipoglycans (LPS) and β-glucans as well as certain viral proteins can enhance viral resistance in shrimp (Song *et al*., 1997; Huang *et al*., 1999; Takahashi *et al*., 2000; Chang *et al*., 2003; Witteveldt *et al*., 2004). It is also has long been suggested that the sequence-independence induction of antiviral immunity by dsRNA merely exists in vertebrates. This might be true, however, there is ample evidence on the degradation of endogenous RNA as the result of sequence specific effects of dsRNA in many invertebrates (Robalino *et al*., 2004). For instance, Robalino *et al*. (2004) and He *et al*. (2005) provided optimism through their experiments using commercially important shrimp, *Litopenaeus vannamei* and *Penaeus japonicus*, respectively. In fact, under experimental conditions, the marine shrimp, *L. vannamei* surprisingly activated the innate antiviral response, similar to that of vertebrates' by recognising dsRNA as a virus-associated molecular pattern (Robalino *et al*., 2004). Three modes of crustacean antiviral immunity that actively battled two different major shrimp viruses, the *Taura Syndrome Virus* (TSV) and the *White Spot Syndrome Virus* (WSSV) were explored through the injection of dsRNA which significantly induced shrimp resistance, similar to that is conferred by mammal IFN type I (Robalino *et al*., 2004). The induction of this antiviral state was dsRNA-sequence independent and was therefore discrete from the sequence-specific dsRNA-mediated interference phenomenon (RNAi), providing the first evidence of the occurrence of an inducible nonspecific antiviral state in crustacea, indicating that interferon-like proteins, similar to that of vertebrates, could exist in shrimps.

It has been suggested that there was a similarity of 40% between the unique clone of interferon like protein (IntIP) from experimentally WSSV-infected *P. japonicus* and IFN-α of sheep, mouse, and monkey by using a combination of suppression subtractive hybridization (SSH) and differential hybridization that was also used to profile gene expression of WSSV-resistant shrimp of *P. japonicus* (He *et al*., 2005). However, the interpretation of these results requires careful scrutiny. First, it does not explain the IntIP antiviral activity and its expression in WSSV-resistant shrimps and did not confirm the IntIP (one clone) in the haemocytes of naive shrimps of *P. japonicus* (Rossa & Barracco, 2008). Secondly, the deposited sequences in GenBank completely differed to any interferon or IFN conserved domains and to any other known protein because the deposited 5'3'open reading frame (ORF) of their presumed IntIP from *P. japonicus* without a larger sequence containing 5' and 3' adjacent regions. In addition,the alignment between *P. japonicus* IntIP and mammalian interferon was poorly described. For instance, the analysis of IntIP sequence in GenBank database by using *Basic Local Alignment Search Tool-x* (BLASTx) program resulted in a translated nucleotide sequence (5'3' frame 3) strongly matched (60%-73% identity) with mitochondrial olygomycin binding fraction- adenosine triphosphate synthase-beta chain (F_o-ATP synthase–β chain) of insects (Rosa & Barracco, 2008). There was also no expression of IntIP in the haemocytes the naive *P. japonicus* (Rosa & Barracco, 2008).

The recombinant IntIP was suggested to have significant power to decrease the cytopathic effect (CPE) caused by Singapore grouper iridovirus (SGIV) on grouper embryonated egg (GP) cells (EC_{50} = 10 µg/mL) and was not cytotoxic to the fish cells CC_{50} > 200 µg/mL (He *et al*., 2005). Although this may in fact occur, the reason why antiviral activity of IntIP and its' expression only in WSSV-resistant *P. japonicus* is still not understood even though the expression of the recombinant, the antiviral activity of *P. japonicus* IntIP on SGIV-GP cell lines was examined (Rosa & Barracco, 2008). Finally, the differential expression of a four clones 2'5'- oligoadenylate synthetase (OAS), in the WSSV-infected *P. japonicus* which was claimed has 32% identity with that of mouse-OAS was unconfirmed because the sequences were neither reported nor deposited in the GenBank. Therefore, it is probable that interferon like molecule of *P. japonicus* highly corresponds to a partial sequence of mitochondrial F_o-ATP synthase–β chain, constitutively expressed, and not to an induced-IFN (Rosa & Barracco, 2008).

CONCLUSION

The existence of interferon in vertebrates is definite. On the other hand, the role of IFN in invertebrates is still unknown. The evidence indicates that invertebrates such as insects and crustacean possess interferon-like molecules as their immune systems specifically deal with pathogen invaders. However, how widely spread of interferon immune response inducer or interferon-like molecules in these groups of animals is an important issue that remains to be explored.

Apart from the ambiguity in the findings of He *et al*. (2005), to date, no other successful identification of the homolog-IFN in crustacea such as shrimps, has been made Indeed, the fact that gene sequences homolog to vertebrate interferon were absent in the available complete genomes of protostomes, including the animal class of insects and crustaceans also supports the idea of a lack of interferon or IFN-like molecules in shrimp (Rosa & Barracco, 2008).

Thus, it is crucial to stress that a nonspecific antiviral state in shrimps can be efficiently triggered by both dsRNA and siRNA, leading to a rational consideration that anonymous cytokines, with no apparent identity to vertebrate IFNs are present in shrimps and are responsible for antiviral protection. In addition to this, the recent finding of identical vertebrate interferon response through Janus kinase (JAK)-like molecules and signal tranducer as well as activator of transcription (STAT)-like molecules in insects sheds new light to the invertebrate immune system. Optimistically, these immune proteins can be identified in a near future and after exposing the molecular sources of their induction and routes, they could spawn a better approach in to the management of shrimp viral diseases in the aquaculture systems (Rosa & Barracco, 2008).

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