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Post-translational modifications and regulations of RLR signaling molecules in cytokines-mediated response in fish

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ABSTRACT

Teleosts rely on innate immunity to recognize and defense against pathogenic microorganisms. RIG-I-like receptor (RLR) family is the major pattern recognition receptor (PRR) to detect RNA viruses. After recognition of viral RNA components, these cytosolic sensors activate downstream signaling cascades to induce the expression of type I interferons (IFNs) and other cytokines firing antiviral responses. Meanwhile, numerous molecules take part in the complex regulation of RLR signals by various methods, such as post-translational modification (PTM), to produce an immune response that is appropriately balanced. In this review, we summarize our recent understanding of PTMs and other regulatory proteins in modulating RLR signaling pathway, which is helpful for systematically studying the regulatory mechanism of antiviral innate immunity of teleost fish.

1. Introduction

The invasion of numerous harmful microorganisms poses a persistent threat to organisms. Vertebrates rely on their innate and adaptive immunity to fight off intruders (Thaiss et al., 2016). The innate immune system is responsible for triggering an immediate immunological response (Lazarte et al., 2019). Its activation requires the recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) (Broz and Monack, 2013). PRRs are a set of membrane or cytosol sensors, including toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide oligomerization domain-like receptors (NLRs), C-type lectin receptors (CLRs) and cytosolic DNA sensors (Poynter et al., 2015).

In mammals, RNA viruses can be recognized by endosomal Toll-like receptors or cytosolic RLR sensors. The RLR family is composed of three members: RIG-I, melanoma differentiation associated factor 5 (MDA5), and laboratory of genetics and physiology-2 (LGP2) (Rehwinkel and Gack, 2020). Upon recognition of viruses, RIG-I and MDA5 recruit the downstream adaptor mitochondrial antiviral signaling (MAVS) through the CARDs. The interactions between RIG-I/MDA5 and MAVS convert MAVS from inactive, autoinhibited monomer into prion-like oligomers, which is the symbol of the activated MAVS. The activated MAVS further recruits and transmits signals to IκB kinase ε (IKKε) and tank-binding kinase 1 (TBK1). Afterward, the activated TBK1 phosphorylates IFN-regulatory factor 3/7, which then induces the production of interferons (Liu et al., 2015; Wu and Hur, 2015).

Type I IFNs are secretory cytokines and exert autocrine and/or paracrine effects. They bind to their receptors (interferon-α/β receptor (IFNAR)) and subsequentially activate the JAK-STAT pathway. STAT1 and STAT2 are the hallmarks of the JAK-STAT pathway. After phosphorylation, these two proteins combine with IRF9 to generate the transcriptional complex known as interferon-stimulated gene factor 3 (ISGF3). The compound enters the nucleus and attaches to the IFN-stimulated response element (ISRE) of IFN-stimulated genes (ISGs), initiating the transcription of ISGs. Ultimately, the host establishes the antiviral state against viral infection (Owen et al., 2019).

In the last few years, more and more studies on teleost fishes have revealed that there is a certain conservatism between the innate immunity of fishes and that of mammals. For example, fishes also rely on PRRs to detect viral nucleic acids and trigger antiviral responses by activating the IFN system. Besides, the innate immune response in fish is also tightly controlled by lots of mechanisms for a balanced antiviral defense, including post-translational modification (PTM) by regulatory enzymes or regulation by associated proteins (Chang, 2021). This review examines the recent progress in the molecular regulation of RLR signal transduction in the teleost, with an emphasis on the post-translational modification (PTM) of the key molecules involved in this pathway.

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2. Regulation by post-translational modification

2.1. Phosphorylation-mediated regulation of RLR signaling

Phosphorylation is a reversible PTM of proteins. When a protein is catalyzed by a kinase, the serine (Ser), threonine (Thr), or tyrosine (Tyr) residues of the substrate are modified by the addition of the phosphate groups, resulting in significant protein conformational changes, which finally affect the properties or function of the protein (Hunter, 2012). Nowadays, phosphorylation and dephosphorylation have received increased attention in the modulation of RLR signaling pathway in teleost (Fig. 1).

2.1.1. Phosphorylation of RIG-I

In resting mammalian cells, phosphorylation of RIG-I keeps this protein in an inactive state. Following viral infection, RIG-I needs to be dephosphorylated by PP1A (Rehwinkel and Gack, 2020). Fathead minnow (*Pimephales promelas*) PP1A shares a high amino acid sequence identity with human PP1A. The activity of fathead minnow RIG-I is dramatically increased by the coexpression of PP1A, indicating that the dephosphorylation of RIG-I by the phosphatase in fish is also essential for its activation (Jami et al., 2021).

2.1.2. Phosphorylation of TBK1

As a crucial kinase in the transmission of RLR signals, TBK1 can form signaling complexes with MAVS, TRIF, or STING. Thereafter, TBK1 in this signalosome phosphorylates IRF3 to further promote the expressions of type I IFNs. In this process, the activity of TBK1 is tightly regulated by elaborate regulations, such as phosphorylation and dephosphorylation. In common carp (*Cyprinus carpio*), TICAM-1 is reported to interact with TBK1 and augment its phosphorylation, facilitating the production of antiviral cytokines (Liu et al., 2021). Additionally, in grass carp (*Ctenopharyngodon idella*), CiSRC, a tyrosine kinase, interacts with CiTBK1 to promote the phosphorylation of CiTBK1, which in turn enhances the downstream IFN signaling (Lv et al., 2021).

On the contrary, a set of studies claimed that the activity of TBK1 is impaired by dephosphorylation. In grass carp, cell division cycle-25a (Cdc25a) interacts with CiTBK1 and prevents the synthesis of IFN1 by dephosphorylating CiTBK1 at Ser172 (Deng et al., 2021). Furthermore, in zebrafish (*Danio rerio*), signal transducer and activator of transcription 6 (STAT6) reportedly interacts with TBK1, causing dephosphorylation of TBK1 and impairing its kinase activity (Li et al., 2017).

2.1.3. Phosphorylation of IRF3 and IRF7

IRF3 and IRF7 are members of the IRF family and are key transcription factors that activate the IFN genes. The activation of IRF3/7 needs to be phosphorylated. In grass carp, RNASEK was found to increase the phosphorylation of IRF3/7 and was identified as a novel modulator for boosting IFN secretion (Sun et al., 2021). Besides, it has been revealed that grass carp GPATCH3 (G-Patch Domain-Containing Protein 3) serves as an activator in STING-IRF3 mediated signaling. In mechanism, the phosphorylation and the nuclear translocation of CiIRF7 were promoted by the overexpression of CiGPATCH3 and CiSTING, respectively (Li et al., 2020a).

Dephosphorylation usually functions to inhibit IRF3/IRF7-mediated antiviral activity. Grass carp NIMA (never in mitosis, gene A)-related kinase 6 (CiNEK6) interacts with CiIRF3 after poly I:C challenge and decreases the phosphorylation of CiIRF3 (Xu et al., 2020). In zebrafish, ovarian tumor domain-containing 6B (OTUD6B) attenuates the capacity of TBK1 to associate with IRF3 and IRF7, leading to the inhibition of the phosphorylation of these two transcription factors (Zhou et al., 2021c). In addition, zebrafish FGFR3 decreases the phosphorylation of IRF3 mediated by TBK1, which restricts the induction of IFN (Liu et al., 2019). In crucian carp, FTRCA1 is a negative regulator which mediates TBK1 degradation, thereby reducing the phosphorylation of IRF3 (Wu et al.,

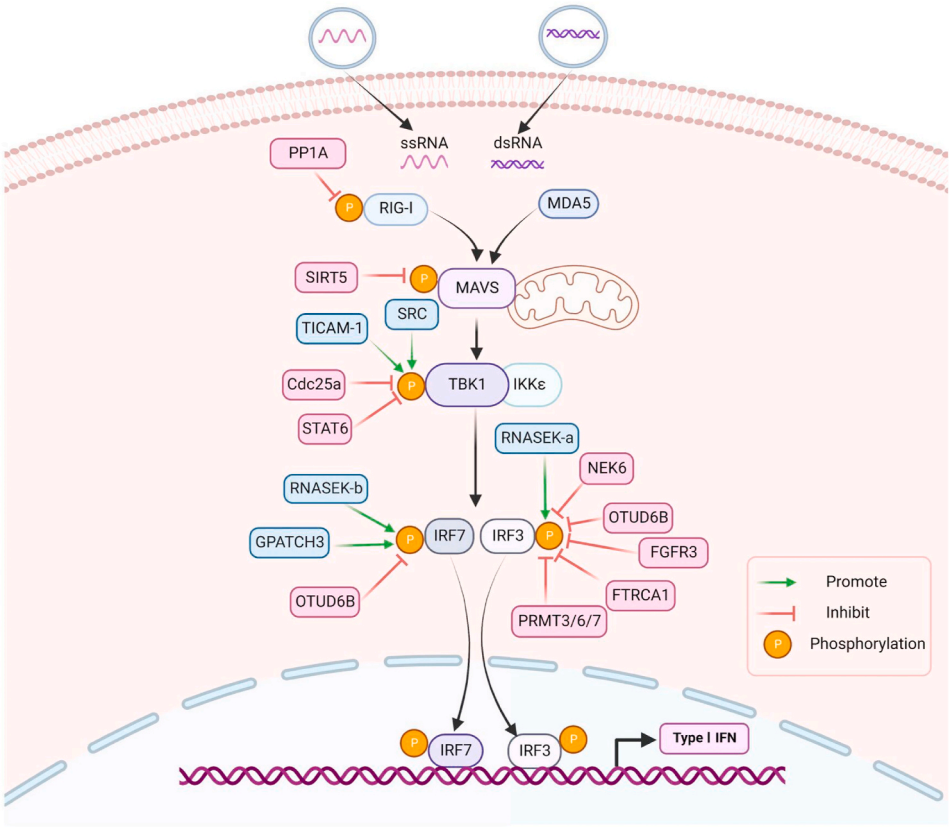

Fig. 1. The regulation of RLR signaling pathway by phosphorylation in teleost fishes.
In fathead minnow, following recognition of viral RNA, RIG-I was dephosphorylated by PP1A which facilitates its activation. Common carp TICAM-1 and grass carp SRC promote TBK1 phosphorylation, while grass carp Cdc25a and zebrafish STAT6 inhibit TBK1 phosphorylation. Zebrafish SIRT5 suppresses the phosphorylation of MAVS. Grass carp RNASEK and GPATCH3 augment the phosphorylation of IRF3 or IRF7, while grass carp NEK6, PRMT6, zebrafish OTUD6B, FGFR3, PRMT3, PRMT7, and crucian carp FTRCA1 inhibit the phosphorylation of IRF3.

2019). Protein arginine methyltransferases (PRMTs) can transfer a methyl group from S-adenosyl-methionine to an arginine guanidine nitrogen (Blanc and Richard, 2017). In grass carp, CiPRMT6 suppresses the phosphorylation of IRF3 after binding to CiIRF3 in a competitive manner (Jiang et al., 2022a,b). In zebrafish, both PRMT7 and PRMT3 suppress cellular antiviral response against viral infection and also impair the phosphorylation of IRF3 (Zhu et al., 2020a, 2020b).

2.2. Ubiquitination-mediated regulation of RLR signaling

Ubiquitin is a 76 amino acids protein, which can form polyubiquitin chains at the lysine residues (K6, K11, K27, K29, K33, K48, and K63) or the N-terminal methionine residue (M1) (Swatek and Komander, 2016). Protein ubiquitination is a dynamic multifaceted post-translational modification catalyzed by three kinds of enzymes: ubiquitin-activating (E1) enzymes, ubiquitin-conjugating (E2) enzymes, and ubiquitin-ligating (E3) enzymes, leading to different destinies of the target proteins (Akutsu et al., 2016). K48-linked ubiquitin chains usually target proteins for proteasome degradation, while K63-linked ubiquitination often facilitates signal transduction (Zhou et al., 2017). Extensive studies have shown that ubiquitination also regulates RLR signaling in fish (Fig. 2).

2.2.1. Ubiquitination of RIG-I

Fish RIG-I, similar to mammalian RIG-I, its activity is strictly controlled by ubiquitination. In zebrafish, TRIM25 interacts with the N- and C-terminal domains of RIG-I and induces the K63 polyubiquitination of this sensor, which is vital for its activation (Jin et al., 2019). Another study demonstrated that zebrafish RNF135 interacts with zbRIG-I to facilitate its K63-linked ubiquitination, thereby enhancing its downstream IFN signaling (Lai et al., 2019). In grass carp and crucian carp (*Carassius auratus*), the cyclic GMP-AMP synthase (cGAS) blocks RIG-I-mediated IFN response, which is achieved by inhibiting the K63-linked ubiquitination of RIG-I and its ability to bind with MAVS (Zhou et al., 2021a). In addition, grass carp Mex3A is a recently discovered inhibitor in IFN signaling. This protein binds to RIG-I and aids in its ubiquitination and destruction (Jiang et al., 2022a,b). On the contrary, viruses have evolved escaping strategies to evade host antiviral immune responses, such as GCRV. GCRV can employ VP4 for evading the host's innate immune response. This viral protein is able to bind to RIG-I and promote its K48-linked ubiquitination, which induces RIG-I to be degraded by the proteasome (Su et al., 2020). In addition, VP56 was found to combine with VP4 in the cytosol to expedite the degradation of RIG-I and make it easier for GCRV to evade detection (Su et al., 2021).

2.2.2. Ubiquitination of MAVS

Many studies have reported that ubiquitination is crucial for the control of interferon signaling that is mediated by piscine MAVS. In zebrafish, transmembrane protein 33 (TMEM33) has been discovered to function as a suppressor in MAVS-mediated IFN signaling. Overexpression of this ER membrane protein facilitates the K48 polyubiquitination of MAVS and leads to the degradation of MAVS (Lu et al., 2021b). Similarly, zebrafish VHL tumor suppressor protein (pVHL) is another inhibitor of MAVS that can promote its proteasomal degradation. pVHL enhanced the K48-linked ubiquitination of the wild-type MAVS but did not affect its mutant (K420R), suggesting the amino acid residue at position 420 of MAVS is the pivotal site for pVHL modification (Du et al., 2015). Another recently discovered inhibitor of zebrafish MAVS is SIRT5. This protein adversely regulates the IFN signaling by weakening the phosphorylation and ubiquitination of MAVS (D.D. Chen et al., 2022). In sea perch (*Lateolabrax japonicus*), it is revealed that ring finger protein 114 (LjRNF114) serves as a repressor of MAVS-mediated antiviral response against RGNNV. This E3 ubiquitin ligase in sea perch targets MAVS and TRAF3 to promote their K27- and K48-linked ubiquitination and induces their degradation (Xiang et al.,

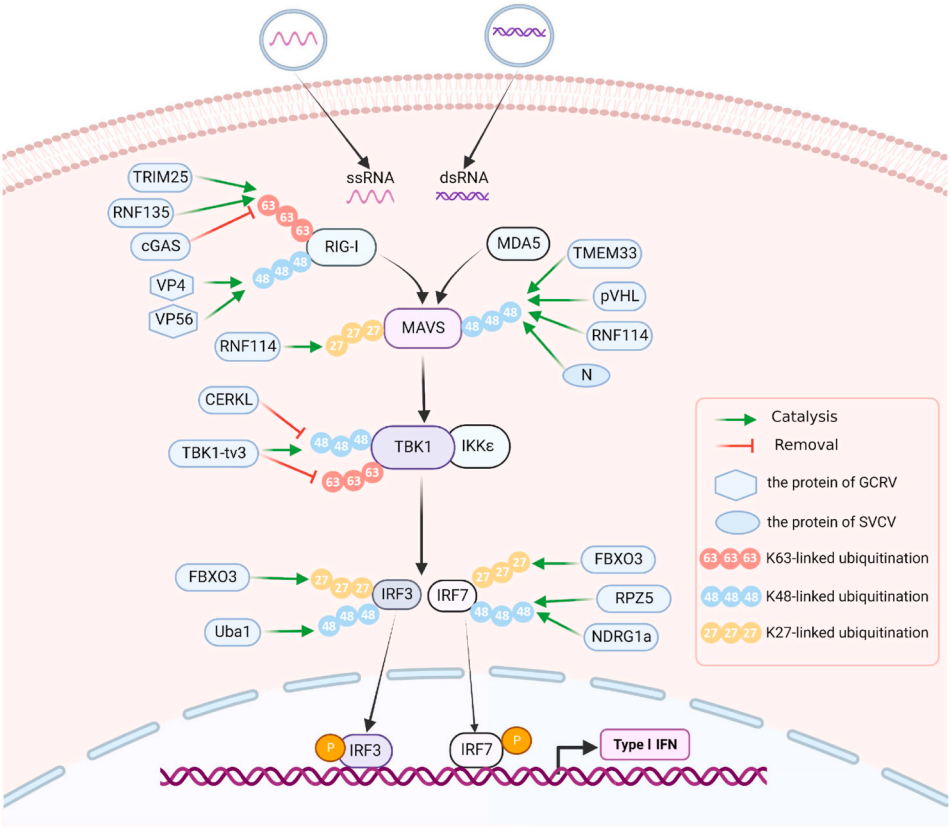

Fig. 2. The regulation of teleost RLR signaling pathway by ubiquitination.
Zebrafish TRIM25 and RNF135 promote the K63-linked ubiquitination RIG-I and facilitate its activation, while grass carp and crucian carp cGAS inhibit its K63-linked ubiquitination. Zebrafish TMEM33, pVHL, and sea perch RNF114 are negative regulators that target MAVS and promote its K48-linked ubiquitination. Zebrafish TBK1-tv3 negatively regulates TBK1 by promoting its K48-linked ubiquitination or inhibiting K63-linked ubiquitination, but CERKL can enhance TBK1 stability by impairing its K48-linked ubiquitination. Zebrafish FBXO3, Uba1, RPZ5 and NDRG1a degrade IRF3 or IRF7 by promoting their K27- or K48- linked ubiquitination. In addition, the viral proteins SVCV N and GCRV VP4/VP56 are able to promote K48-linked ubiquitination of MAVS and RIG-I respectively, facilitating their proteasomal degradation.

2021). In addition to the host proteins, viral proteins can also regulate MAVS activity. For instance, spring viremia of carp virus (SVCV) has evolved mechanisms to escape from the host antiviral response. The N protein of SVCV reportedly targets zebrafish MAVS for K48-linked ubiquitination, thereby reducing its protein level (Lu et al., 2016).

2.2.3. Ubiquitination of TBK1

Ubiquitination of piscine TBK1 is essential for host defense against viral infection. A recent study showed that zebrafish ceramide kinase-like (CERKL) is a positive regulator of the host's antiviral innate immune response. On the one hand, it can target TBK1 and block its K48-linked ubiquitination, thereby enhancing TBK1 protein stability. On the other hand, it can degrade the SVCV P protein by reducing its K63-linked ubiquitination, thereby limiting viral replication (X. Chen et al., 2022).

Ubiquitination of piscine TBK1 is also important in maintaining immune homeostasis. In zebrafish, TBK1-tv3, one of the TBK1 spliced isoforms, impedes the formation of TBK1 dimerization and promotes TBK1 degradation via two distinct mechanisms: on the one hand, it facilitates TBK1 proteasomal degradation by catalyzing K48-linked ubiquitination, on the other hand, it might act as a deubiquitinase, interrupting K63-linked polyubiquitination of TBK1 (Zhang et al., 2020). In addition, GCRV has evolved mechanisms that hijack TBK1 to escape host antiviral signaling activation. In grass carp, low concentrations of GCRV tends to impede the K63-linked ubiquitination of TBK1 and induce degradation of TBK1 by promoting K48-linked ubiquitination (Rao et al., 2019).

2.2.4. Ubiquitination of IRF3 and IRF7

Several investigations in zebrafish have demonstrated that certain molecules dampen the antiviral immune response by modulating the ubiquitination of IRF3 or IRF7. For instance, zebrafish ovarian tumor domain-containing 6B (OTUD6B) is able to interact with IRF3/7 and diminish the TRAF6-mediated K63-linked polyubiquitination of these two molecules (Zhou et al., 2021c). In addition, zebrafish FBXO3, a member of the F-box family, interacts with IRF3/7 and particularly catalyzes K27-linked ubiquitination of IRF3/7, leading to their degradation (Li et al., 2020b). Moreover, zebrafish Ub-activating enzyme (Uba1) interacts with IRF3 and degrades IRF3 via the K48-linked ubiquitination to prevent unrestrained IFN production (Chen et al., 2021a). Zebrafish rapunzel 5 (RPZ5) protein stimulates the degradation of phosphorylated IRF7 via K48-linked ubiquitination, preventing uncontrolled IFN secretion (Lu et al., 2019b). Similarly, zebrafish NDRG1a has been discovered as a new regulator in IFN immune balance, which also targets IRF7 and promotes the K48-linked ubiquitination and degradation of IRF7 (Lu et al., 2019a).

2.3. Acetylation-mediated regulation of RLR signaling

2.3.1. Acetylation of IRF3 and IRF7

Compared with phosphorylation and ubiquitination, there are few studies on acetylation in teleost. Briefly, it is a modification mediated by acetyltransferases that leads to the addition of an acetyl group on the target protein. A recent study reported that acetylation can influence the functions of grass carp IRF3/7. Grass carp KAT8 (CiKAT8) has been characterized as an acetyltransferase. This enzyme can target IRF3/IRF7 and enhance their acetylation, which affects the affinity of IRF3/7 with ISRE response elements, thereby suppressing the downstream antiviral signaling (Li et al., 2021).

3. Regulation by autophagy (protein degradation)

Autophagy is a highly conserved mechanism that can degrade redundant, defective, or malfunctioning organelles and proteins. Accumulating evidence demonstrates that autophagy can regulate excessive IFN production in fish, such as by targeting the key molecules in RLR signaling pathway (Fig. 3).

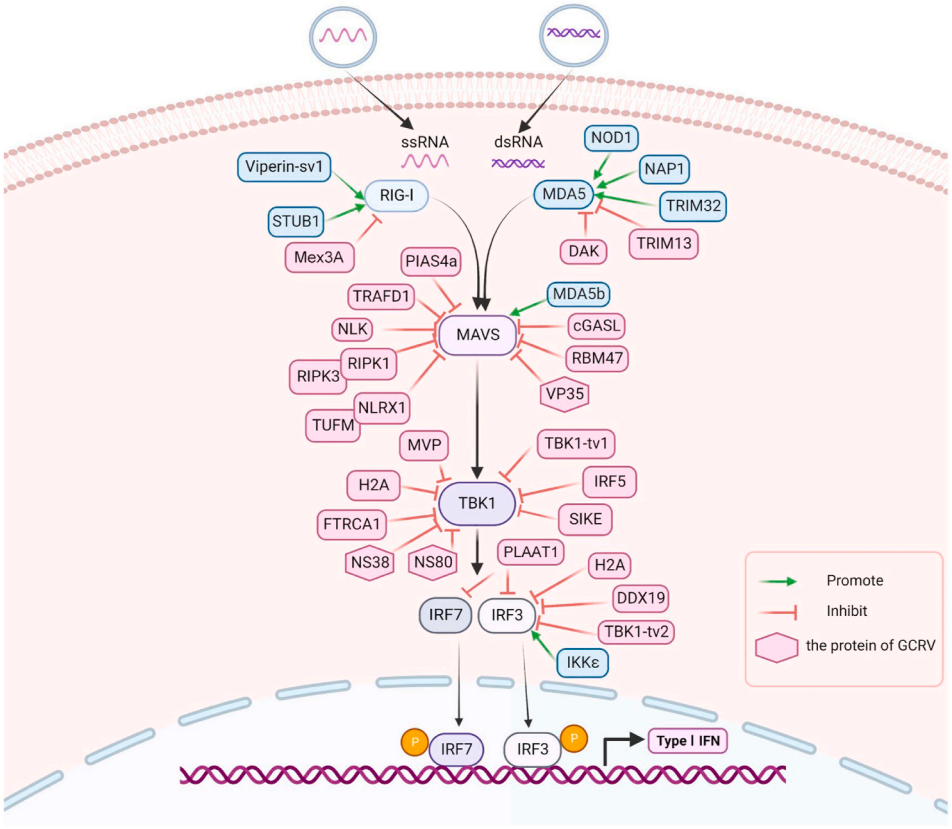

Fig. 3. The regulation of teleost RLR signaling pathway by interacting proteins.
The activities of RLR-IRF3/7 signaling axis are positively or negatively regulated by interacting proteins that modulate their expression (or degradation), dimerization (or oligomerization), formation of functional complexes, or subcellular localization. For example, fathead minnow viperin_sv1 and zebrafish STUB1 are positive regulators which promote the expression of RIG-I, while zebrafish Mex3A promotes the degradation of RIG-I. Zebrafish TBK1_tv1 and TBK1_tv2 disrupt the formation of a functional TBK1-IRF3 complex. Zebrafish PLAAT1 is the suppressor of IRF3/7 by facilitating their degradation. Black carp DDX19 restricts the nuclear translocation of IRF3 to suppress downstream signaling. In addition to host factors, NS38 and NS80 of GCRV inhibit the formation of functional TBK1-containing complexes to restrict downstream signaling.

3.1. Protein degradation of MAVS

In grass carp, cyclic GMP-AMP synthase-like (cGASL) is evolutionarily closest to mammalian cGAS. MAVS protein level is reduced when this protein is overexpressed. However, this phenomenon can be blocked by autophagy inhibitor and autolysosome inhibitor, indicating that cGASL degrades MAVS through the autophagy-lysosome pathway (Zhou et al., 2021b). In zebrafish, RNA-binding motif protein 47 (RBM47) has been discovered as a suppressor of MAVS, which also mediates the degradation of MAVS in the manner described above (Lu et al., 2020). In addition, viruses can also employ autophagy to evade the host immune response. The VP35 protein of GCRV can interact with zebrafish MAVS and degrade it in an autophagy manner (Lu et al., 2021a).

3.2. Protein degradation of TBK1

Another molecule in zebrafish, major vault protein (MVP) has been identified as a suppressor targetting TBK1. It interacts with TBK1 and mediates the lysosome-dependent degradation of TBK1 to inhibit the host immune response (Li et al., 2019b). In addition, it is reported that zebrafish Histone H2A targets TBK1 and IRF3 to promote their degradation via the lysosomal pathway and impairs the formation of TBK1-IRF3 functional complex (Wu et al., 2021). Besides, in crucian carp, FTRCA1, a species-specific member of finTRIM family, is associated with TBK1 to trigger autophagy-lysosomal degradation of TBK1 (Wu et al., 2019b).

4. Regulation by interacting proteins

A recent series of studies demonstrated that many RLR-binding proteins play different roles in the positive or negative regulation of RLR signaling pathway in fish (Fig. 3).

4.1. RIG-I-interacting proteins

In fathead minnow, a new splice variant of virus inhibitory protein (viperin_sv1) has been identified as a positive regulator of RIG-I. This protein interacts with RIG-I and increases its protein stability, thereby enhancing RIG-I-mediated antiviral signaling (Gao et al., 2021). In zebrafish, STIP1 homology and U-box containing protein 1(STUB1) reportedly promotes the protein expression of RIG-I for activating RIG-I signaling, but the mechanism still needs to be further studied (Shi et al., 2022).

4.2. MDA5-interacting proteins

In zebrafish, two MDA5 variants have been discovered. The shorter form (MDA5b) functions as a positive regulator in MDA5/MAVS/IFN signaling (Zou et al., 2014). Similarly, zebrafish NOD1 is another positive modulator in this signaling. It can significantly strengthen the association between MDA5a and MAVS (Wu et al., 2020). In black carp, NAK-associated protein 1 (NAP1) interacts with bcMDA5 and boosts its downstream antiviral signaling. On the other hand, black carp dihydroxyacetone kinase (bcDAK) functions as a negative regulator of bcMDA5 which interacts with bcMDA5 to block the downstream signaling (Cai et al., 2020; Liao et al., 2022). The function of MDA5 is also regulated by tripartite motif (TRIM) family members. In orange-spotted grouper, TRIM13 is a negative regulator of MDA5, while TRIM32 can significantly enhance the MDA5-mediated interferon immune response (Huang et al., 2016; Yu et al., 2017).

4.3. MAVS-interacting proteins

Several proteins have been identified as negative regulators of MAVS. In black carp, NOD-like receptor (NLR) family member X1 (bcNLRX1) interacts with bcMAVS to adversely regulate bcMAVS-mediated antiviral signaling through its NACHT domain (Song et al., 2019); black carp receptor-interacting serine/threonine protein kinase 1 (bcRIPK1) interacts with bcMAVS to inhibit host immune response against RNA viruses (Xie et al., 2020). Besides, bcTUFM and bcRIPK3 have been identified as the binding proteins to NLRX1 and RIPK1 respectively, which play a synergistic role in the negative regulation of MAVS/IFN signaling by NLRX1 and RIPK1 (Cao et al., 2021; Dai et al., 2021). Moreover, black carp NLK and TRAFD1 also have been identified as association proteins of MAVS, which act as suppressors in RLR signaling through inhibiting MAVS (Chen et al., 2021b; Yan et al., 2020). In zebrafish, PIAS4a downregulates IFN signaling and impedes MAVS-mediated antiviral response (Xiong et al., 2012).

4.4. TBK1-interacting proteins

TBK1 is a core kinase in RLR signaling. The formation of functional TBK1-containing complexes, including MAVS, TBK1, IKK, IRF3, IRF7, STING, TRIF, TRAF, and other proteins, is critical for TBK1 activity. In zebrafish, the isoforms of TBK1 named TBK1_tv1 and TBK1_tv2 compete with TBK1 and IRF3 to prevent the development of a functional TBK1-IRF3 complex. Besides, since TBK1 enzymatic activation requires dimerization or higher-order oligomerization, TBK1-tv3 has been reported to impair TBK1 dimerization and thus negatively regulate its downstream signals (Hu et al., 2018; Zhang et al., 2020). In black carp, bcIRF5 adversely controls bcTBK1-mediated antiviral response in healthy cells; however, after virus infection, it correlates with bcTBK1 and causes cell death to restrict the virus propagation (Yang et al., 2019). Furthermore, black carp SIKE has been identified as a suppressor which interacts with bcTBK1 in inhibiting bcTBK1-mediated antiviral response (Li et al., 2019a). In addition to host factors, a recent report on GCRV showed that the nonstructural proteins NS38 and NS80 of GCRV-873 interact with zebrafish TBK1, inhibit the formation of functional TBK1-containing complexes to suppress host IFN production (Zhang et al., 2022a).

4.5. IRF3- and IRF7-interacting proteins

In zebrafish, PLAAT1 is a newly identified suppressor in the host antiviral immune response which can interact with IRF3 and IRF7 and facilitates their degradation (Zhao et al., 2022). In black carp, IκB kinase ε (bcIKKε) is a positive regulator which targets bcIRF3 and boosts antiviral signaling (Wang et al., 2021). In contrast to bcIKKε, black carp DExD/H-box RNA helicase 19 (bcDDX19) is an antagonist of cellular antiviral defense. Mechanistically, it binds with bcIRF3 and limits its nuclear translocation to block downstream signaling (Liu et al., 2022). Another molecule, transforming growth factor-β activated kinase 1 (TAK1), is a member of the mitogen-activated protein kinase family. In different fish, this protein seems to use different mechanisms to regulate IRF3/7-mediated IFN signal. In black carp, bcTAK1 is a positive regulator, it dramatically improves bcIRF7-mediated antiviral response (Wang et al., 2019). However, in grouper, TAK1 suppresses IRF3- or IRF7-mediated signaling, indicating that TAK1 seems to utilize different mechanisms in regulating IRF3/7 mediated IFN signal in different fish (Zhang et al., 2022b).

5. Conclusions and perspectives

Interferons play important roles in the antiviral immune response in teleost fish. In this process, interferon production is regulated by numerous host enzymes, including E3 ligase, deubiquitinase, kinase, phosphatase, etc., which modify key innate signaling molecules. This review summarizes the recent developments in the regulation of RLR signaling pathway by PTMs and other regulatory proteins, which is beneficial for methodically exploring the regulatory mechanism of antiviral innate defenses in teleost fish. We think the following aspects are intriguing or merit further study.

Firstly, with the progress in mass spectrometry (LC-MS/MS) and proteomics, most of the molecular mechanisms of immune regulation have been studied in human cells, and more than 200 different types of PTMs have been identified. However, the function of unconventional modifications such as acetylation, deacylation, glycosylation, methylation, and SUMOylation has not been well elucidated, especially in fish. Therefore, we can employ these technologies to aid in our systematic and deeper exploration of the regulatory mechanisms of PTMs in fish.

Secondly, we found that the functions of some regulatory proteins are different in fish and higher vertebrates. For example, in mammals, cGAS is a positive regulator in the host antiviral response. However, the cGAS of grass carp and crucian carp functions as a negative regulator in the RLR signaling (Zhou et al., 2021a). Furthermore, even the same protein functions differently in various species. For instance, TAK1 in black carp is a positive regulator in the innate immune response against GCRV (Wang et al., 2019). However, in grouper, this protein facilitates the replication of SGIV (Zhang et al., 2022b). Therefore, paying attention to the proteins with different functions in different species is valuable for us to explore the distinctions between the innate immune regulatory mechanisms of lower vertebrates and higher vertebrates from the perspective of comparative immunology.

Thirdly, aquatic viruses have evolved lots of strategies against the host antiviral immune response. For example, the N protein of SVCV targets MAVS and degrades MAVS (Lu et al., 2016). Besides, the NS38 and NS80 of GCRV interact with TBK1 to suppress its downstream IFN signaling (Zhang et al., 2022a). Therefore, we can further explore virus-encoding proteins and the proteins that serve as their targets in the host and analyze their relationships and regulatory mechanisms. This will give us a crucial theoretical basis for us to explore the prevention and control strategies of aquatic viruses.

In summary, the knowledge of post-translational modifications and regulations in fish innate immunity will help us to develop drugs, vaccines, or adjuvants that are conducive to enhancing the activity of antiviral factors or blocking virus antagonism, and creative tactics for preventing and curing fish diseases.

Data availability

Data will be made available on request.

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