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Molecular identification and functional characterization of IRAK-3 from a teleost fish, the orange-spotted grouper (*Epinephelus coioides*)

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ABSTRACT

Interleukin-1 receptor-associated kinase-3 (IRAK-3) is a unique IRAK family member, which negatively regulates the TLR-mediated immune response in mammals. However, the function of IRAK-3 remains to be elucidated in fish. In the present study, an IRAK-3 cDNA sequence (EcIRAK-3) with an ORF of 1776 bp encoding 591 amino acids was identified in the orange-spotted grouper (*Epinephelus coioides*). Sequence analysis indicated that EcIRAK-3 shared the conserved structure characteristics and functional sites of vertebrate IRAK-3, and has a high sequence identity and phylogenetic relationship with that of other fish species. The genomic EcIRAK-3 ORF contained 13 exons and 12 introns, which was similar to that of most other fish species. In healthy grouper, EcIRAK-3 was ubiquitously expressed in seven tested tissues with the highest expression in the gills. Following *Cryptocaryon irritans* infection, the EcIRAK-3 transcript was up-regulated in the gills during the course of the experiment, but down-regulated in the spleen at an earlier point in time. EcIRAK-3 was localized in both the cytoplasm and nucleus in a condensed form, and its cellular distribution was affected by the death domain and ProST domain. In addition, EcIRAK-3 significantly increased MyD88-mediated NF-κB activity, and its function was ProST domain and kinase domain dependent. Taken together, the results obtained here have contributed to the understanding of the function of IRAK-3 in fish.

1. Introduction

The Interleukin-1 receptor-associated kinase (IRAK) family members are important signal molecules, which are involved in both Toll-like receptor (TLR) and interleukin-1 (IL-1R) signal pathways [1]. In mammals, four members, IRAK-1, IRAK-2, IRAK-3, and IRAK-4, have been identified [2–5]. All IRAKs share conserved structural features, comprising an *N*-terminal death domain, a ProST domain, a central kinase domain, and a *C*-terminal domain (except IRAK-4). Among them, IRAK-1 and IRAK-4 have kinase activity, but IRAK-2 and IRAK-3 lack kinase activity, which may be due to a change of the aspartate residue in the IRAK kinase domain to an asparagine residue in IRAK-2 or serine in IRAK-3 [6]. However, the importance of their kinase activity or the adaptor role of IRAK-1 and IRAK-4 is controversial in mammals.

IRAK-3 (IRAK-M) was originally identified as an activator of NF-κB, which was predominately expressed in monocytes and macrophages in humans, but also in B cells in mouse [4,7]. However, further studies have demonstrated that IRAK-3 was a negative regulator in the TLR signal pathway, which inhibited the dissociation of IRAK-1 and IRAK-4 from MyD88 to form an IRAK1–TRAF6 complex, and thereby prevents downstream signaling [8,9]. Su et al. have indicated that IRAK-3 is selective in the negative regulation of p38 activation but not ERK or JNK activation, and inhibits the alternative NF-κB pathway but not the classical pathway induced by TLR2 ligand Pam_3CSK_4 [10,11]. Recently, Zhou et al. have reported that IRAK-3 was able to form a MyD88/IRAK-4/IRAK-3 complex, which mediated MEKK3-dependent NF-κB activity and subsequently induced the expression of inhibitory molecules such as SOCS1, SHIP1, and A20 [12]. In addition, IRAK-3 inhibits the TLR7-mediated production of cytokines and chemokines, implying that IRAK-3 adopts a more active manner to negatively regulate the inflammation response induced by TLRs.

In fish, three IRAK family members, IRAK-1, IRAK-3, and IRAK-4, were identified in a variety of species, but IRAK-2 was only found in the *Latimeria chalumnae* genome. Our and other studies have indicated that

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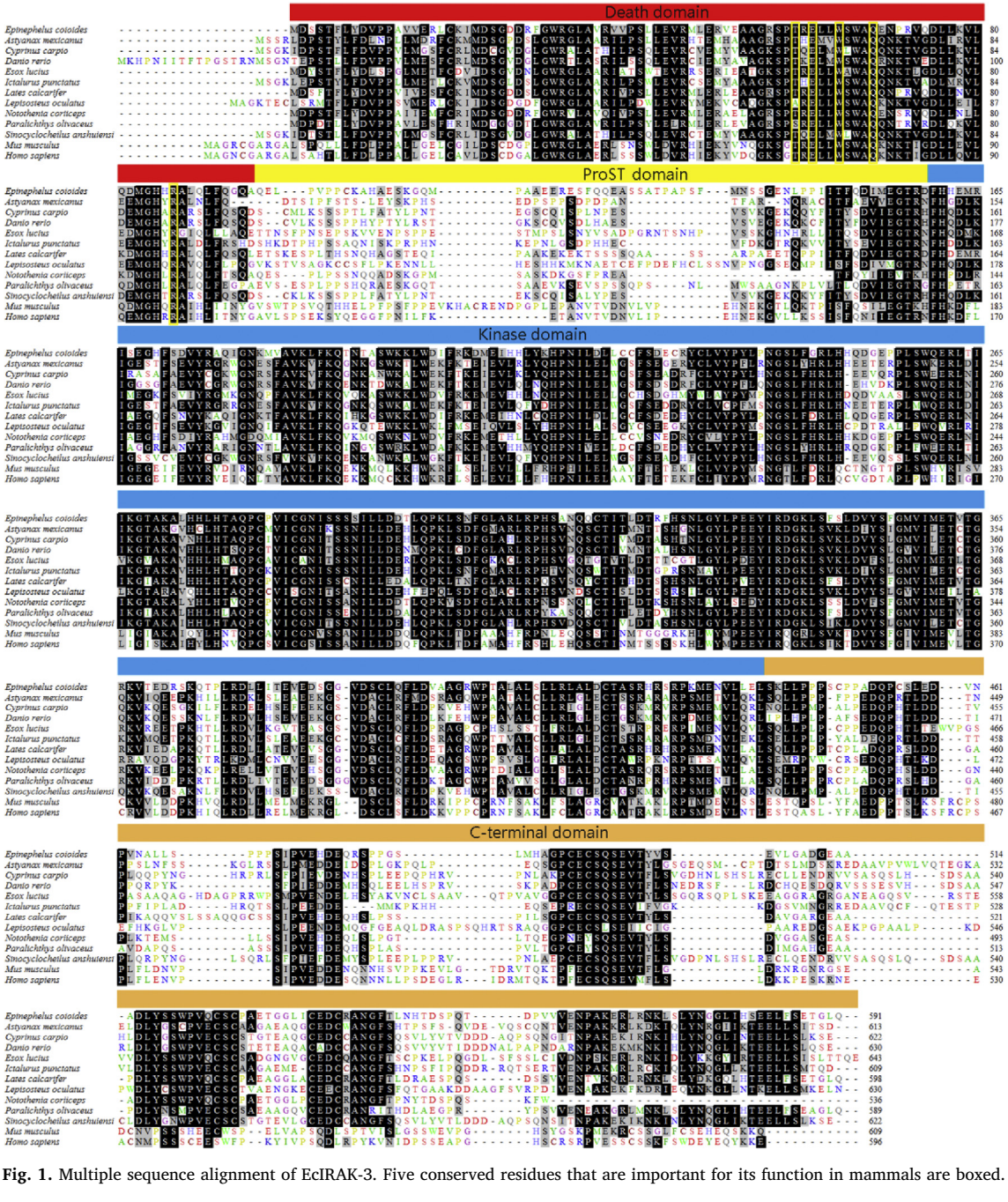

Fig. 1. Multiple sequence alignment of EcIRAK-3. Five conserved residues that are important for its function in mammals are boxed.

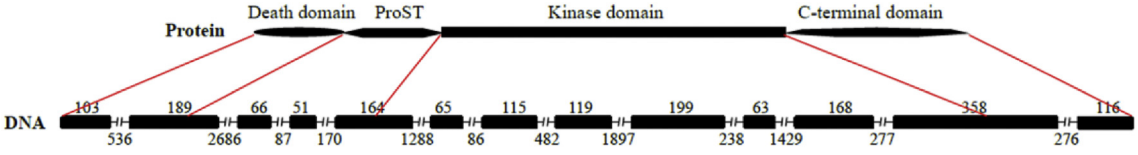

Fig. 2. The protein and genomic structure of EcIRAK-3. The boxes and the lines between the boxes indicate exons and introns, respectively, and the numbers indicate the sizes (bp) of each exon and intron.

piscine IRAK-1 could induce NF-κB activity, but IRAK-4 significantly impaired TLR2 or MyD88-mediated NF-κB activity [13–17]. Although piscine IRAK-3 sequence could be retrieved from the NCBI GenBank database, the function of IRAK-3 remains unclear in fish. Therefore, to investigate the role of piscine IRAK-3, herein, an IRAK-3 cDNA (EcIRAK-3) and genomic sequence were identified in the orange-spotted grouper (*Epinephelus coioides*), and the sequence characteristics were analyzed. Additionally, the expression pattern of EcIRAK-3 in healthy and *Cryptocaryon irritans* infected grouper was investigated using real-time PCR. Finally, the signaling role of full-length and domain deletion mutants of EcIRAK-3 was assessed.