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Cloning of an orange-spotted grouper (*Epinephelus coioides*) Mx cDNA and characterisation of its expression in response to nodavirus

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Abstract

Molecular cloning and nucleotide sequencing of cDNA encoding an orange-spotted grouper (*Epinephelus coioides*) homolog of *Mx* (''*OsgMx*'') was conducted and its possible role in fish immunity was analysed. Similar to mammalian Mx, the *OsgMx* are members of a family of interferon-inducible genes that are expressed by cells in response to nodavirus and iridovirus naturally-infected. Expression of *OsgMx* mRNA was noticeably upregulated in all tissues by nodavirus naturally-infected grouper. The transcription of *OsgMx* gene increased 6 h after intramuscular injection of nodavirus experimentally-infected fish and peaked at 72 h in their brains. Analysis of the 5′-flanking sequence of the gene shows that as in pufferfish and zebrafish, the *OsgMx* promoter contains two potential interferon-stimulated response element (ISRE) responsible for the induction of interferon-inducer polyinosinic-polycytidylic acid (Poly[I:C]). Transient transfection of grouper cells in *gfp*-reporter gene assays shows that the activation of the grouper Mx promoter fragment by Poly[I:C] is sufficient to allow the expression of green fluorescent protein (GFP). These results may provide a possible regulated pathway against nodavirus.

Keywords: Orange-spotted grouper; Mx protein; Interferon-stimulated response element (ISRE); Nodavirus

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1. Introduction

Piscine Mx is an IFN-induced cytoplasmic protein [1] with antiviral activity against a number of RNA viruses including orthomyxoviruses [2] and rhabdovirus. It belongs to a family of conserved large GTPases [3] and to the superfamily of dynamin-like GTPases [4], which are both involved in a wide range of intracellular transport processes [5]. These GTPases represent force-generating enzymes that use energy from GTP hydrolysis to induce conformational changes required for their mechanochemical function [6].

Recently the cDNA of *Mx* has been cloned and characterised in certain fish: rainbow trout [7,8], Atlantic salmon [9], Japanese flounder [10], Atlantic halibut [11], pufferfish [12], gilthead sea bream [13], channel catfish [14], goldfish [15], and zebrafish [16]. Each Mx protein contains the tripartite GTP-binding motifs which bind and hydrolyze GTP for antiviral function [17]. Adjacent to the conserved tripartite GTP-binding motifs, Mx proteins have a second highly conserved region that is also found in the *Drosophila* biological dynamin domains. The dynamin superfamily of GTPase is involved in endocytosis and vesicle transport [18]. In addition, since leucine zippers have been implicated in the dimerisation of a number of proteins including the Jun-Fos heterodimerisation, the polymerisation of human Mx has been attributed to the leucine zipper motif in the carboxy terminal end of all Mx proteins [19].

In fish, the function of these *Mx* genes remains to be elucidated, especially their role in the enhancement of natural resistance against viral infection. It has been shown that the three different types of Mx in trout have no apparent antiviral activity against rhabdovirus and infectious hematopoietic necrosis virus (IHNV) [8]. Recently, it has been established that Japanese flounder Mx proteins have antiviral function as has been found to be the case in higher organisms [20]. In Atlantic salmon, its Mx1 protein has been found to inhibit the virus-induced cytopathic effect (CPE), viral protein synthesis, and transcription of viral RNA [21]. The interferon of zebrafish cell line (ZF4), which constitutionally expressed zebrafish interferon cDNA, has been shown to significantly inhibit against a fish rhabdovirus [22]. These results indicate that piscine interferon establishes an antiviral state in cells via the Mx proteins as does mammalian Mx. Over the years, piscine nodavirus has devastated the grouper (*Epinephelus* spp) culture industry in Taiwan and other Asian countries and a better understanding of fish natural defense mechanisms against such pathogens is needed. It is hoped that knowledge of grouper Mx protein will contribute to the understanding gene function associated with interferon-signaling molecules in grouper. Furthermore, future studies are needed to identify all the molecular components of the interferon system, which cause the unanticipated high sensitivity of grouper to protection against nodavirus, the major pathogen for the grouper culture industry.

Over the past years, piscine nodavirus, a member of the *Betanodavirdae* family, is the causative agent of viral nervous necrosis or fish encephalitis that produces high mortalities in hatchery-reared larvae and juveniles of marine fishes in Taiwan, Japan, Australia, and Europe [23,24]. This virus is an unenveloped, icosahedral capsid (25–30 nm in diameter), and the genome is composed of bipartite, single-stranded, positive-sense RNA molecules [25]. Betanodavirus is neuropathogenic and inflicts conspicuous damage characterised by vacuolation and degeneration of neurons throughout the central nervous system [26]. However, infection in older red-spotted grouper and adults are generally mild and often go unrecognised suggesting that adult groupers have an efficient defense mechanism against nodaviruses. Mechanism of the piscine interferon protection against nodavirus at the molecular level is not well understood and the well studied interferon-inducible antiviral proteins, such as double-stranded RNA-dependent protein kinase, *Mx* and ribonuclease L, have not been studied in cultured grouper. Here, the evidence that OsgMx in grouper can respond to the nodavirus to induce *Mx* transcription is presented, and we also show that the grouper *Mx* promoter contained the ISRE element and responded to Poly[I:C].