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Reprint of: Virus-binding proteins and their roles in shrimp innate immunity☆

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ABSTRACT

Disease outbreaks caused by viral pathogens constitute a major limitation to development of the shrimp aquaculture industry. Many research have been conducted to better understand how host shrimp respond to viral infections with the aim of using the gained knowledge to develop better strategies for disease management and control. One approach has been to study the interactions between host and viral proteins, and particularly host virus-binding proteins that might play an important role in the viral infection process. Within the past five years, increasing numbers of virus-binding proteins (VBPs) have been reported in shrimp. Characterization of these molecules has emphasized on their potential therapeutic applications by demonstrating their activities in inhibition of viral replication via *in vivo* neutralization assay. However, signaling to induce innate antiviral immune responses as a consequence of binding between viral proteins and VBPs remain to be fully elucidated.

1. Introduction

In mammals, effective antiviral innate immune responses are triggered when invading viruses are detected by immune system receptors leading to the initiation of protein signaling pathways that induce mechanisms that control infections [1,2]. The receptors are called “pattern recognition receptors (PRRs)” that can be grouped into 3 classes including secreted or soluble PRRs, membrane-bound PRRs and cytoplasmic PRRs. A number of soluble receptors for human viruses such as poliovirus [3], rhinovirus [4], human immunodeficiency virus [5], hepatitis B virus [6] and influenza A virus [7] have been described and may have potential interest as antiviral agents *in vivo*. In shrimp, many virus-binding proteins have been identified, but their relationship to signaling pathways involved in immunity is still unclear. In addition, the reported shrimp proteins do not recognize common patterns among viral proteins and thus it might not fit the criteria of pattern recognition proteins (PRPs) or pattern recognition receptors (PRRs) such as those that interact with unique bacterial and fungal cell-wall components. In this review, we prefer to use the more general expression “virus-binding proteins (VBPs)” until their roles in innate immunity have been fully elucidated. Shrimp VBPs reported so far are mainly soluble proteins, such as lectins, and membrane-bound proteins, such as integrin and the *Penaeus monodon* chitin-binding protein (PmCBP). Cytoplasmic proteins which play a major role in the recognition of viral nucleic acids have been less frequently reported [8].

2. Viral proteins

To date, more than twenty viruses have been reported to infect penaeid shrimp. They include both DNA viruses such as monodon baculovirus (MBV), white-spot syndrome virus (WSSV), hepatopancreatic parvovirus (HPV) and infectious hypodermal and hematopoeitic virus (IHHNV), and RNA viruses such as yellow-head virus (YHV), Taura syndrome virus (TSV) and Laem-Singh virus (LSNV) [9]. Among them, WSSV, YHV and TSV are highly virulent to penaeid shrimp [10].

2.1. WSSV proteins

WSSV has been classified as the only member of the new family *Nimaviridae*, genus *Whispovirus* [11,12]. It is a large, enveloped, ovaloid DNA virus with a flagellum-like tail and a nucleocapsid

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consisting of stacked rings. The ~300 kbp viral genome contains at least 181 ORF, most of which encode polypeptides with no detectable homology to other known proteins [13,14]. WSSV has a broad host range since more than 43 species of arthropods have been reported as hosts or carriers. The extensive host range and wide tissue tropism imply that the cellular receptor for WSSV is conserved and ubiquitous. A number of WSSV-binding proteins (WBPs) have been reported to bind to viral particles or viral protein components. Those binding to WSSV structural proteins have been most extensively studied. By using proteomic approaches, more than 40 structural proteins of WSSV have been identified, including envelope proteins (VP19, VP28, VP31, VP36B, VP38A, VP51B, VP53A), tegumental proteins (VP26, VP36A, VP39A, VP95) and nucleocapsid proteins (VP664, VP51C, VP60B, VP15) [15,16]. The interactions among the WSSV structural proteins have been determined by using coimmunoprecipitation and yeast two-hybrid assays [17]. These interactions were then used to construct a 3D model of the membrane protein complex and the nucleocapsid of WSSV. In the model, four envelope proteins VP28, VP51A, VP19 and VP37 are predicted to expose on the external surface of the virion, and they are therefore very likely play important roles in infection by binding to cell receptors or by fusing with the host membrane. Yi et al. (2004) showed that VP28 is necessary for the attachment and penetration of WSSV into shrimp cells [18]. Both VP19 and VP51A might have similar functions. VP37 has been previously shown to be able to attach to shrimp cell membranes, probably through its RGD motif [19]. VP24 has been reported to interact with VP28 and to be involved in virus infection [20]. By using the biotin label transfer technique and far-Western blot, direct interaction between VP26 and the nucleocapsid protein VP51C was revealed, suggesting that VP26 acts as a linker protein to bridge the envelope to the nucleocapsid protein of the virus [21]. Another nucleocapsid protein, VP15 might function as a DNA-binding protein, since VP15 is a very basic protein that resembles histone proteins [22]. Neutralizing antibodies raised against VP281, VP446, VP19 and VP28 were able to protect shrimp against virus infection [23,24]. Schematic diagram of WSSV proteins that interact with shrimp binding proteins is shown in Fig. 1.

2.2. YHV proteins

Yellow-head virus (YHV) is a rod-shaped, single stranded, positive-sense RNA virus that has a spiked envelope with a genome of approximately 27 kb. YHV has been assigned to a new family *Roniviridae*, genus *Okavirus* [25]. YHV is one of a complex of six closely related viruses infecting *P. monodon* shrimp [26]. Only YHV type-1 is known to be highly virulent and it has been reported so far only from Thailand. Other penaeid and palemonid shrimp species have been shown to be susceptible to experimental infection with YHV type-1 or its less virulent relatives but *P. monodon* appears to be a major natural host [27]. Three YHV structural proteins have been described, including two-envelope glycoproteins gp116 and gp64 and a nucleocapsid protein p20 [28,29]. The nature of the glycans attached to N-linked sites in the mature gp116 and gp64 glycoproteins has been determined [30]. Of the three YHV structural proteins, the amino acid sequence of gp116 is most variable amongst the genotypic variants [26,31,32]. Electron microscopy has shown that gp116 forms the prominent envelope surface projections on mature virions [33]. Although both gp116 and gp64 are suspected to play crucial roles in cellular binding and entry, only antibodies to gp116 (not gp64) inhibit virus infection [34,35]. Indeed, a shrimp cell-surface receptor has been identified that binds gp116 and is purported to mediate cell attachment [35].

2.3. TSV proteins

Taura syndrome virus (TSV) is a small, naked (+) ssRNA virus with a 10 kb genome and it is currently classified as an unassigned species in the family *Dicistroviridae* [36–38]. It has caused severe disease outbreaks only with farmed *Penaeus (Litopenaeus) vannamei* and its susceptible host range is far more restricted than that of WSSV. Its genome is characterized by two large open reading frames (ORF). ORF1 encodes its putative non-structural proteins and ORF2 encodes its structural proteins. The predicted amino acid sequence of ORF1 revealed sequence motifs characteristic of non-structural proteins of a helicase, a protease and an RNA-dependent RNA polymerase. ORF2 encodes three capsid proteins of 55, 40 and 24 kDa called VP1, VP2 and VP3, respectively [39].

3. Virus-binding proteins (VBPs)

3.1. WSSV-binding proteins

Within the past five years, several secreted and cell-surface VBPs have been reported from shrimp (Table 1). Of these,

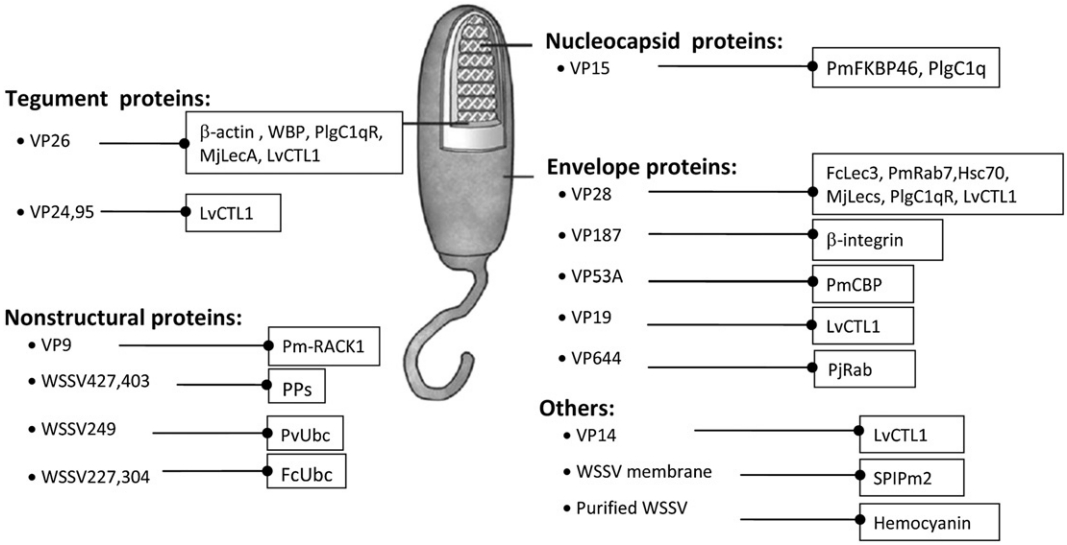

Fig. 1. Schematic diagram of WSSV proteins that interact with shrimp binding proteins [modified from Ref. [16]].