Developmental and Comparative Immunology 59 (2016) 177–185

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

WSV399, a viral tegument protein, interacts with the shrimp protein *Pm*VRP15 to facilitate viral trafficking and assembly

Phattarunda Jaree [a], Saengchan Senapin [b, c], Ikuo Hirono [d], Chu-Fang Lo [e], Anchalee Tassanakajon [a], Kunlaya Somboonwiwat [a, *]

[a] Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Phayathai Rd., Bangkok 10300, Thailand
[b] Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand
[c] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani 12120, Thailand
[d] Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan
[e] Institute of Bioinformatics and Biosignal Transduction, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan, ROC

ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form
26 January 2016
Accepted 27 January 2016
Available online 29 January 2016

Keywords:
Penaeus monodon
White spot syndrome virus
Host-viral interaction
*Pm*VRP15
WSV399

ABSTRACT

Viral responsive protein 15 (*Pm*VRP15) has been identified as a highly up-regulated gene in the hemocyte of white spot syndrome virus (WSSV)-infected shrimp *Penaeus monodon*. However, the function of *Pm*VRP15 in host–viral interaction was still unclear. To elucidate *Pm*VRP15 function, the interacting partner of *Pm*VRP15 from WSSV was screened by yeast two-hybrid assay and then confirmed by co-immunoprecipitation (Co-IP). Only WSV399 protein was identified as a *Pm*VRP15 binding protein; however, the function of WSV399 has not been characterized. Localization of WSV399 on the WSSV virion was revealed by immunoblotting analysis (*in vitro*) and immunoelectron microscopy (*in vivo*). The results showed that WSV399 is a structural protein of the WSSV virion and is particularly located on the tegument. Gene silencing of *wsv399* in WSSV-infected shrimp reduced the percentage of cumulative mortality by 74%, although the expression level of a viral replication marker gene, *vp28*, was not changed suggesting that WSV399 might not involved in viral replication but viral assembly. Because it has already been known that tegument proteins function in capsid transport during viral trafficking and assembly, interaction between *Pm*VRP15 on hemocyte nuclear membrane and the WSV399 viral tegument protein suggests that *Pm*VRP15 might be required for trafficking and assembly of WSSV during infection.

1. Introduction

White spot syndrome virus is one of the most severe shrimp pathogens. WSSV-infected shrimp population can reach a cumulative mortality of 100% within 3–10 days (Durand et al. 1997). The WSSV virion contains a rod-shaped nucleocapsid, typically measuring 65–70 nm in diameter and 210–350 nm in length. The nucleocapsids, which contain a DNA-protein core bound by a distinctive capsid layer giving it a cross-hatched appearance, are wrapped singly into an envelope to shape the virion (Durand et al. 1997; Nadala and Loh, 1998). About 40 WSSV proteins have been characterized (Escobedo-Bonilla et al. 2008). Most of these proteins are structural proteins in the envelope (VP12B, VP13B, VP14, VP19, VP28, VP31, VP32, VP33, VP38A, VP39B, VP41A, VP41B, VP51A, VP51B, VP53A, VP60A, VP90, VP110, VP124, VP180 and VP187), tegument (VP12A, VP26, VP36A, VP39A and VP95) and nucleocapsid (VP15, VP24, VP35, VP51C, VP60B, VP75, VP76, VP136A, VP190 and VP664). Some WSSV proteins that act as non-structural proteins are probably involved in transcriptional regulation (VP9), virus proliferation (WSV021) and regulation of DNA replication (WSV477). To date, many WSSV proteins are still uncharacterized.

Several WSSV-binding proteins have been identified in shrimp but their roles in signaling pathways related to immunity are still unclear. Viral-binding proteins are categorized into two types base on interaction with the virus: non-specific interaction and specific interaction. Non-specific interacting proteins like hemocyanin can bind to WSSV virion and act as antiviral factors of *Penaeus monodon* (Zhang et al. 2004). Meanwhile, many WSSV-binding proteins with specific interactions between host and viral protein have been reported. For example, the interaction between a major nucleocapsid

* Corresponding author.
E-mail address: kunlaya.s@chula.ac.th (K. Somboonwiwat).

http://dx.doi.org/10.1016/j.dci.2016.01.020
0145-305X/

protein of WSSV like VP15 with PmFKBP46 has been described and thought to be involved in genome packaging during virion assembly (Sangsuriya et al. 2011). Interaction between VP26, a major tegument protein of WSSV, with shrimp cytoskeletal protein β-actin and 3 kDa WSSV-binding protein (WBP) was identified (Xie and Yang, 2005, Liu et al. 2011; Youtong et al. 2011) and its important role in WSSV infection was also revealed. The endosomal protein from the hemocytes of *P. monodon*, PmRab7, was identified as VP28-binding protein (Sritunyalucksana et al. 2006). PmRab7 gene silencing resulted in the decrease in WSSV replication suggesting that PmRab7 functions as an important regulator of intracellular trafficking (Ongvarrasopone et al. 2008). PlgC1qR, a receptor for globular head domain of complement component C1q, could bind to VP15, VP26, and VP28 of WSSV and possibly play a role in controlling antiviral mechanism (Watthanasurorot et al. 2010).

From our previous report (Vatanavicharn et al. 2014), a viral responsive gene, *Pm*VRP15 that is highly abundant in WSSV-infected shrimp, was identified by suppression subtractive hybridization. *Pm*VRP15 encodes for a deduced 137 amino acid protein containing a putative transmembrane helix and located around the nuclear membrane in shrimp hemocyte. The silencing of *Pm*VRP15 gene in *P. monodon* significantly decreased viral gene expression and cumulative mortality suggesting its role in WSSV propagation pathway. Moreover, *Pm*VRP15 also called *Pm*ERP15 was found to be involved in endoplasmic reticulum (ER) stress triggered by WSSV infection. From the results, *Pm*ERP15 was induced by ER stress and located in the ER. After silencing *Pm*ERP15 in WSSV-infected shrimp, the viral copy number did not change in the shrimp gill while the cumulative mortality was lower than control group. It was concluded that although *Pm*ERP15 was not involved in WSSV replication, it was essential for survival of WSSV-infected shrimp (Leu et al. 2015).

Despite the previously mentioned studies, the function of *Pm*VRP15 protein was still unclear. To characterize the involvement of *Pm*VRP15 in WSSV infection in shrimp, the interactions between *Pm*VRP15 and viral proteins, were identified in this study. Also, the implication of the *Pm*VRP15-binding protein in viral infection was revealed.

2. Materials and methods

2.1. Construction of bait plasmids containing the N-, C- terminus and open reading frame (ORF) of PmVRP15 gene

For yeast two-hybrid screening, three *Pm*VRP15 bait vector containing ORF, N-terminus fragment, and C-terminus fragment, were constructed. Firstly, the open reading frame (ORF), N- and C-terminus fragment of *Pm*VRP15 gene were amplified by PCR using gene specific primers (Table 1). The PCR conditions were 94 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, and then a final extension at 72 °C for 5 min using RBC *Taq* polymerase (RBC Bioscience). The PCR products were analyzed by 1.5% (w/v) agarose gel electrophoresis and purified using Nucleospin® Extract II kit (Macherey–Nagel). The purified PCR products were double-digested with restriction enzymes and cloned in-frame into pGBKT7 vector, a bait vector, cut with the same restriction enzymes and transformed into an *Escherichia coli* XL1-blue and then selected on LB agar plate containing 30 μg/ml kanamycin. The recombinant plasmids were subjected to nucleotide sequencing to verify the sequences of inserts (Macrogen Inc., Korea).

2.2. Yeast two-hybrid screening

Yeast two-hybrid library construction and screening were performed. *Saccharomyces cerevisiae* AH109 was co-transformed with pGBKT7-*Pm*VRP15, pGBKT7-N-ter*Pm*VRP15, or pGBKT7-C-ter*Pm*VRP15 (bait vectors) and the prey pGADT7-WSSV ORF library (Sangsuriya et al. 2014). The transformants were selected on a synthetic defined (SD) medium lacking leucine and tryptophan (SD/-Leu/-Trp). Interaction between the bait and prey fusion proteins was indicated by the growth and blue color change on the double dropout SD/-Leu/-Trp media containing X-alpha-Gal (DDO/X). In order to ascertain that the positive clones contained the interacting proteins, the positive blue colonies were subsequently grown on a higher stringency quadruple dropout medium lacking leucine, tryptophan, adenine and histidine (SD/-Leu/-Trp/-Ade/-His) but containing X-alpha-Gal (QDO/X). Prey plasmids were then isolated from those positive clones that had all four yeast reporter genes [ADE2, HIS3, MEL1 (encodes for α-galactosidase) and lac Z (encodes for β-galactosidase)] activated, transformed into *E. coli* XL1-Blue to recover the plasmid, and subjected to DNA sequencing. The obtained sequences were searched against the NCBI GenBank database using BLASTX to identify the clones. To confirm the screening results, recovered prey plasmids were co-transformed with the bait plasmid into *S. cerevisiae* AH109 and plated onto QDO/X medium according to the manufacturer's instructions (BD Biosciences).

2.3. Production and purification of recombinant PmVRP15 protein and WSSV protein

The single colony of *E. coli* stain C43 (DE3) containing recombinant plasmid pET22b-*Pm*VRP15 (a gift from Assist. Prof. Dr. Kuakarun Krusong) was cultured in LB-amp medium with shaking at 250 rpm at 37 °C for overnight as a starter. The overnight culture was inoculated into fresh LB-amp medium until OD_{600} reached 0.5 then induced with 1 mM IPTG for 1 h to over-produce the r*Pm*VRP15. The cell pellet was collected, resuspended in 50 mM Tris–HCl, pH 7.0 and sonicated with a Bransonic 32 (Bandelin). The soluble fraction containing r*Pm*VRP15 was collected by centrifugation at 10,000 rpm for 20 min, and further centrifuged to isolate the membrane protein part of *E. coli* that contain r*Pm*VRP15 by ultracentrifugation at 100,000 × g for 1 h. The resulting pellet was homogenized in ice-cold solubilization buffer (50 mM Tris–HCl, pH 7.0, 20 mM Immidazole, 300 mM NaCl, 1% dodecyl-L-D- maltoside (DM) and 20% glycerol) at 4 °C for overnight. The crude supernatant of r*Pm*VRP15 protein was finally collected by ultracentrifugation at 100,000 × g for 30 min and subjected to purification through Ni-NTA column (GE healthcare). Crude protein was loaded onto the Ni-bead column pre-equilibrated in the equilibration buffer (50 mM Tris–HCl, pH 7.0, 20 mM Imidazole, 0.1% DM and 10% glycerol) and incubated at 4 °C for 2 h. The column was washed with washing buffer (50 mM Tris–HCl, pH 7.0, 50 mM Imidazole, 0.1% DM and 5% glycerol) and r*Pm*VRP15 was eluted by elution buffer (50 mM Tris–HCl, pH 7.0, 300 mM Immidazole, 0.1% DM and 5% glycerol). The elution fraction was subjected to Amicon® Ultra-4 Centrifugal Filter Units cut-off 3 kDa (Millipore) to concentrate and exchange buffer to 10 mM Tris–HCl, pH 7.0, 0.07% DM and 2.5% glycerol. The purified protein was analyzed using 15% SDS-PAGE. The protein concentration was determined using the Bradford method.

For recombinant WSV399 protein production, *wsv399* gene was amplified by PCR using gene specific primers with *Xho*I and *Eco*RI restriction sites, WSV399-*Xho*I-F and WSV399-*Eco*RI-R (Table 1). The PCR conditions were 94 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, and then a final