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Short communication

An update on mechanism of entry of white spot syndrome virus into shrimps

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ABSTRACT

Host-parasite relationships can be best understood at the level of protein-protein interaction between host and pathogen. Such interactions are instrumental in understanding the important stages of life cycle of pathogen such as adsorption of the pathogen on host surface followed by effective entry of pathogen into the host body, movement of the pathogen across the host cytoplasm to reach the host nucleus and replication of the pathogen within the host. White Spot Disease (WSD) is a havoc for shrimps and till date no effective treatment is available against the disease. Moreover information regarding the mechanism of entry of White Spot Syndrome Virus (WSSV) into shrimps, as well as knowledge about the protein interactions occurring between WSSV and shrimp during viral entry are still at very meagre stage. A cumulative and critically assessed information on various viral-shrimp interactions occurring during viral entry can help to understand the exact pathway of entry of WSSV into the shrimp which in turn can be used to device drugs that can stop the entry of virus into the host. In this context, we highlight various WSSV and shrimp proteins that play role in the entry mechanism along with the description of the interaction between host and pathogen proteins.

1. Introduction

White Spot syndrome (WSS) is a highly contagious disease of penaeid shrimps such as *Penaeus monodon*, *Marsupenaeus japonicus*, *Litopenaeus vannamei*, *and Fenneropenaeus indicus* and is caused by White spot syndrome virus (WSSV). Shrimp farming had been practiced originally by the costal people in the South-Asian countries such as Indonesia, Philippines, India, Taiwan, Thailand and Vietnam for food as well as source of revenue for many centuries. Due to the gigantic consumption of shrimps worldwide, shrimp aquaculture eventually began to be practiced globally. The deadliest blow to the shrimp aquaculture industry occurred in 1992 when the most fatal shrimp virus, White spot syndrome virus (WSSV) adversely affected shrimp population [1]. The virus is highly devastating and the deadliest form of virus caused upto 100% shrimp mortality within 3–10 days hence it is considered to be havoc for shrimp population [2]. WSS is the most important scourge for the international shrimp farming commerce [2–6]. Since its emergence the total monetary loss caused by WSD to the shrimp aquaculture industry has been tabulated to be $8–$15 billion [7]. Further, the loss has been estimated to get escalated by $1 billion annually [8,9].

WSSV is a rod-shaped enveloped virus and consists of four components: 300 kbp double-stranded DNA genome, a nucleocapsid, a tegument, and an outer envelope [10,11]. The most essential component of the virion envelop and nucleocapsid are their constituent structural proteins. The basic nature of WSSV, its exact life cycle and mode of infection, mechanisms and strategies used by this virus to infect and replicate in susceptible host cells are also still unknown. Thus understanding the fundamental structural components of both shrimp as well as virus that play role in entry of virus into shrimp becomes highly important. DNA viruses make use of multi-protein complexes for e.g. Herpes virus uses 3 glycoproteins and vaccinia virus uses 8 transmembrane proteins [12] to ingress into the host. Although a precise mechanism of entry of WSSV into host is yet unknown still it is speculated that WSSV, also

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a kind of large enveloped DNA virus, employs multi-protein complex to enter host. In view of this, the present work entails the description of infective proteins of WSSV and receptor proteins of shrimp that are accountable for entry of the virus into the shrimp. Further, it describes some key interactions between shrimp and viral proteins that assist in entry and propagation of virus within the host.

The cannibalistic nature of shrimp is the foremost reason for oral ingestion of dead or WSSV infected shrimp by a healthy shrimp. The digestive tract of shrimp is the primary site of infection. When the viral particles from dead or infected shrimp reach the digestive tract of healthy shrimp, effective binding of viral particle to the host cell surface is vital for instigation of a viral infection. The shrimp digestive tract is composed of the oesophagus, stomach, midgut and hindgut. The oesophagus, stomach and hindgut portions possess a chitinous lining, that is not present in the midgut [13,14]. Instead, the midgut epithelium is generally lined with the peritrophic membrane (PM) which is a semi-permeable, non-cellular membrane surrounding the food bolus in shrimp's gut. The PM is constituted by chitin fibrils embedded in a matrix of proteins, proteoglycans, and mucopolysaccharides [15–17]. Hence, WSSV must traverse through the PM in the midgut or the chitinous lining in the other parts of the digestive tract to cross the basal membranes and ultimately reach the cells of target organs via the circulating plasma. In addition to this, previous studies conducted on the peritrophic membrane in another species of prawn *Sicyonia ingentis* (characteristically much similar to the PM of *L. vannamei*) revealed that only particles smaller than 20 nm could physically permeate across the PM [18]. Since the size and length of WSSV are 70–150 nm (at its broadest point) and 250–380 nm, respectively [19], it is speculated that in order to surpass the underlying basal lamina there must be receptors in the stomach and gut that enable the WSSV to break through the physical barrier of the PM.

Some of the significant receptors that line and form important part of PM are chitin binding proteins (CBPs), Peritrophin-Like Protein (PTs), C Type lectins (CLs) etc. The most studied chitin binding protein is PmCBP (CBP in *Penaeus monodon*). Similar kind of CBP is also present in tissue of *Litopenaeus vannamei*. Yeast two-hybrid experiments revealed that PmCBP could interact with at least 11 WSSV envelope proteins v.i.z VP24, VP53A, VP110, VP53B, VP337, VP32, VP124, VP41A, VP51B, VP60A and VP39B [20]. Additionally, a new viral protein complex was identified that could mediate WSSV entry across the digestive tract by interacting with CBP. The complex was termed 'infectome' and constituted viral proteins viz. VP24, VP28, VP31, VP32 VP39B, VP53A and VP56 [20]. The chitin binding assays further indicated that most crucial interaction among the proteins forming infectome and CBP was between VP24 and PmCBP [21]. As per the Chen's viral membrane model, VP24 is exposed outside of the viral envelope which additionally facilitated its interaction with CBP. Mutagenesis analysis have further depicted that amino acids from 186 to 200 located on the C-terminus region of VP24 interact with CBP. Moreover, the clearance time for ingested food from the entire digestive tract of the shrimp has been reported to be 4 h [22]. Thus within this clearance time, interaction between VP24-chitin is mandatory for the binding of WSSV to the inner surface of the shrimp digestive tract, and failure in the attachment process might lead to futile infection. Due to large dimensions of WSSV it simply cannot infiltrate through the chitinous barriers of digestive tract. Hence, it is quite feasible that after attachment to the chitin layer WSSV recruits a host chitinase to degrade the chitin on the binding site, thereby promoting WSSV to break though the chitin barriers and infect the underlying epithelium. Then, the six envelope proteins (VP28, VP31, VP32, VP39B, VP53A and VP56) with VP53A as the core may anchor to PmCBP on the cell surface and mediate WSSV entry [21]. Apart from having CBP as a crucial receptor in digestive tract of shrimp, some other receptors have also been identified critical for helping the virus intrude into the shrimp especially during the early viral entry stage.

Peritrophin-Like protein in *Litopenaeus vannamei* (LvPT) is another important receptor that is substantially important constituent of the peritrophic membrane and forms receptor to many viral proteins. LvPT is a secretory protein mainly expressed in the stomach, and is the most studied protein among other PTs [23]. Recent studies have recommended the protein to have considerable chitin binding ability and thus contributes to transport of virus across the digestive tract into the circulating plasma. Co-immunoprecipitation confirmed interaction between VP37 and LvPT. An additional Y2H screening and co-immunoprecipitation also identified interactions between LvPT and VP32, VP38A, VP39B, and VP41A. Further, the chitin binding assays also revealed that LvPT-V5 could bind to chitin. Since LvPT is a secretory protein, it is secreted into the plasma of shrimp. The secretion of the receptor protein into the vascular system carries along the viral particles into the vascular system and helps in dissemination of the virions throughout the body [24]. Hence, secretion of LvPT into the vascular system assists the virion particles navigate into the plasma thereby promoting propagation of the virus within the shrimp body.

Another important cell surface protein, named glucose transporter 1 (Glut1), interacts with WSSV envelope protein, VP53A thereby assisting the virions traverse across digestive tract of the shrimp [25,26]. The protein is expressed in muscle, pleopods, epidermis and stomach and Glu1 receptor present on stomach epithelia is assumed to form an important receptor for transporting WSSV across intestinal membrane. Y-2-H and far western blot firmly confirmed that Glut1 could interact both with VP53A and PmCBP. It was further speculated that Glut1 and PmCBP together may form a heterodimer structure which could interact with the WSSV envelope complex formed by the eleven envelope proteins (VP24, VP32, VP39B, VP41A, VP53A, VP53B, VP51B, VP60A, VP110, VP124 and VP337). Glut1 consisted of 6 extracellular regions among which the largest one (named Glut1-loop) had potential to bind WSSV [25]. Far western blot showed that Glut1-loop could interact with VP24, VP28, VP31, VP32, VP39B, VP51B and VP53A [26]. Another important group of receptors that partake in WSSV entry included tetraspanins. Tetraspanins are transmembrane proteins that can form connection between the proteins outside or inside the cell membrane. They belong to the transmembrane 4 superfamily (TM4SF) [27,28]. The most common type of tetraspanins identified in shrimp included, FcTetraspanin-3, FcCD63 and FcCD9 [29]. Out of the three, FcTetraspanin-3 presumably play an indispensable function in response to WSSV infection [30]. FcTetraspanin-3 was detected both in intestine and hepatopancreas by immunohistochemistry studies. The tetraspanin protein constituted four transmembrane domains, a small extracellular loop and a large extracellular loop (LEL) [31]. There were four highly conserved cysteine residues in LEL that formed mushroom-like structure and blocking of the LEL domain of FcTetraspanin-3 could restrain the infection of WSSV [27] although the envelop proteins involved in such interaction are still a matter of investigation.

Other important receptors that are also implicated in viral entry comprised C-type lectins (CLs). Two major CLs identified in shrimp included: MjsvCL designated as *Marsupenaeus japonicus* stomach virus–associated CL [24] and FmCL from *Fenneropenaeus merguiensis*. Usually, the recognition between the host receptors and viral envelope proteins occurs through binding glycans [25,26]. However, proteomic analyses of WSSV structural proteins revealed that none of them were glycosylated [24,27] signifying that the