Shrimp White Spot Syndrome – from Pathology to Pathogenomics

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ABSTRACT—For shrimp with low-level white spot syndrome virus (WSSV, = PRDV) infection, environmental or physiological stresses can trigger replication of the virus and lead to a full-blown outbreak of white spot disease. This review will describe how pathogenomic studies have revealed some of the mechanisms that underlie this aspect of WSSV pathology. In the model that emerges, stressors activate shrimp STAT (signal transducer and activator of transcription), which is then annexed by the virus and used to activate the promoter of the immediate early gene WSSV *ie1*. This in turn leads to rapid replication of the virus.

Key words: white spot syndrome, penaeid acute viremia, pathogenomics, shrimp STAT, WSSV *ie1* gene, WSSV, PRDV

White spot syndrome virus (WSSV), also known as penaeid rod-shaped DNA virus (PRDV), is the type species of the genus *Whispovirus*, family *Nimaviridae* (Vlak *et al.*, 2005; Inouye *et al.*, 1996). It is the causative agent of white spot disease, which has rapid onset and is usually lethal to the host. The virus is extremely virulent (Inouye *et al.*, 1994; Momoyama *et al.*, 1994; Nakano *et al.*, 1994), has a wide host range (Lo *et al.*, 1996; Flegel, 1997) and targets various tissues (Wongteerasupaya *et al.*, 1995; Lo *et al.*, 1997). Replication of the virus is easily triggered by physiological or environmental stress (OIE, 2006; Lo *et al.*, 1997), but partly because no continuous shrimp cell line is currently available, the molecular mechanisms that control WSSV replication and gene transcription still remain unknown. Furthermore, WSSV is very unique, with over 90% of its ORFs showing no similarity to any known proteins (Tsai *et al.*, 2004; 2006; Leu *et al.*, 2009). The complete genome sequence of WSSV is available (WSSV-CN, accession no. AF332093; WSSV-TH, accession no. AF369029; WSSV-TW, accession no. AF440570), and there are now many published shrimp ESTs (O'Leary *et al.*, 2006; Tassanakajon *et al.*, 2006; Leu *et al.*, 2007). However, in absence of functional evidence, the genetic sequence alone cannot fully unravel the molecular mechanisms by which WSSV causes disease. Consequently, in order to understand

the physiological, metabolic, and pathological mechanisms used by WSSV, a global molecular understanding of the virus and its interactions with the host must be developed. This is the field of pathogenomics, which looks at how a pathogen's genetic sequence is related to the mechanisms by which the pathogen causes disease. In this review, we will present a case study showing how rapid replication of the virus is triggered by physiological or environmental stress. We will also show how WSSV genome sequencing was applied to the study of how WSSV causes disease.

We in fact followed two main lines of investigation. We began by screening for WSSV immediate early genes (IE genes), and this was followed by looking at how these IE genes interacted with host genes. This led to the finding that WSSV can use a shrimp protein, STAT (signal transducer and activator of transcription) to activate the WSSV *ie1* promoter. Meanwhile, we also found that WSSV infection, environmental stress and physiological stress, can all activate STAT in shrimp. From all these observations, we inferred that the key to underlying mechanism is that WSSV is able to exploit antiviral STAT in shrimp. From this we were then able to put together our proposed model.

Viral IE genes are very important for viral infection. The expression of viral IE genes depends on the host cell machinery and occurs independently of any viral *de novo* protein synthesis. IE genes are especially important in determining host range. IE gene products may function as regulatory trans-acting factors and may serve to initiate viral replication events during infection (Friesen, 1997).

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To identify WSSV IE genes, protein synthesis had to be inhibited by treating the shrimp with the peptidyl transferase inhibitor cyclohexamide. Since the WSSV genome sequence was known, cDNA microarrays could be constructed, and these were used to screen for WSSV IE genes in the cycloheximide-treated shrimp (Liu *et al.*, 2005). In this way, three WSSV IE genes were identified, with one of these genes, WSSV *ie1*, being of particular interest. Subsequent promoter assay revealed that WSSV *ie1* has a very strong promoter, and that this *ie1* promoter also functions in insect cells (sf-9 cells) (Liu *et al.*, 2005). Like many other immediate early proteins, the WSSV IE1 protein has a transactivation domain; it can form dimers; and it also has DNA binding activity. All of these characteristics suggest that it can act as a transcriptional regulator (Liu *et al.*, 2008). As one of the downstream genes that are regulated by *ie1*, we identified a transcription factor that positively regulates *ie1* itself. Unexpectedly this turned out to be a shrimp STAT (PmSTAT; ie the *Penaeus monodon* STAT). This annexation of PmSTAT by WSSV was unexpected because STAT usually plays a role in host defense (Liu *et al.*, 2007).

The Janus kinase (JAK)-STAT signaling pathway was first identified through studies on cytokines, and in the cytokine-mediated pathway, STATs execute the final step. STAT proteins are a family of transcription factors that are phosphorylated by activated JAK, then dimerize and subsequently translocate to the nucleus, where they transcriptionally activate appropriate genes to mediate various responses, including cell growth, cell differentiation and immune responses (Levy and Darnell, 2002; Shuai and Liu, 2003). In vertebrates, STAT is part of the immune defense system, and it has been shown that STAT has a similar function in invertebrates too. In insect, the first evidence of the JAK/STAT pathway being involved in immune responses was found in the mosquito *Anopheles gambiae* (AgSTAT). The AgSTAT translocated into the nucleus of the fat body cell upon bacterial infection (Barillas-Mury *et al.*, 1999; Lin *et al.*, 2004). *Drosophila* STAT was shown to play a defense role against bacterial infection, and also against viral infection (Agaisse and Perrimon, 2004; Dostert *et al.*, 2005). In light of our previous result, the question then arises: how does WSSV interact with STAT? We found the answer to this question while investigating WSSV *ie1* promoter activity.

WSSV *ie1* has been shown to have a very strong promoter (Liu *et al.*, 2005). Using a dual luciferase assay system, we functionally mapped deletions of the WSSV *ie1* promoter and found that a 23-mer fragment was critical for promoter activity. Sequence analysis of the WSSV *ie1* promoter showed that this 23-mer fragment overlapped with a putative STAT binding motif, and the same dual luciferase assay was used with site-directed mutagenesis to show that this STAT binding site

really was critically important. The STAT binding site located within the -84/-64 region of the WSSV *ie1* promoter, and electrophoretic mobility shift assays (EMSA) of this region with Sf9 cell nuclear extracts showed that the putative STAT binding motif was able to bind with insect STAT to form an (SfSTAT)-DNA complex. EMSA also showed that the same fragment binds with over-expressed PmSTAT in Sf9 cells, and that the recombinant PmSTAT transactivated the WSSV *ie1* promoter in a dose-dependent manner. We further showed that knocking down STAT expression by dsRNA gene silencing reduced the expression level of WSSV *ie1* mRNA (compared to the dsRNA anti-viral effect produced by the non-specific dsRNA control) and led to a reduction in virus copy number (unpublished data). All these data support the conclusion that WSSV successfully annexes a shrimp STAT to enhance the expression of the immediate early gene *ie1* (Liu *et al.*, 2007). But, as described below, WSSV and STAT also interact in another way as well.

It should be noted that STAT transcription is actually reduced after WSSV infection (Chen *et al.*, 2008). However, the levels of activated STAT are increased. After WSSV infection, Western blot analysis using anti-phosphorylated STAT antibody showed an increased level of phosphorylated (activated) STAT in shrimp cells (Liu *et al.*, 2007; Chen *et al.*, 2008), and this activated STAT was translocated from the cytoplasm to the nucleus (Chen *et al.*, 2008). Under normal physiological conditions, microbial infection will activate the host immune-related signaling pathways and produce defense responses. But for WSSV, the outcome is quite different. Like other pathogens, WSSV causes STAT to be activated, and the activated STAT then dimerizes and translocates to the nucleus. However, once in the nucleus, instead of regulating the transcription of host immune genes, the STAT is annexed by WSSV and used to transactivate the promoter of the WSSV *ie1*.

A schematic of the proposed mechanism is shown in Fig. 1. Under stressful conditions, the STAT will be activated and this leads to transactivation of the WSSV *ie1* gene and rapid replication of the virus. Recent results have shown that activation of STAT can be triggered not only by a pathogen, but also by environmental stressors (e.g. hypoxia) and physiological stressors (e.g. spawning) (unpublished data). Under these circumstances, if there is already a low-level WSSV infection, in the cell, then WSSV will exploit the activated STAT and use it to transactivate the WSSV *ie1* gene. This is the mechanism by which stress triggers the rapid replication of the virus.

There are likely to be very many other cellular pathways that are impacted by WSSV infection (Leu *et al.*, 2007; Wang *et al.*, 2007; Wang *et al.*, 2008). Science is very far from understanding the full range of mecha-

Fig. 1. Schematic of the mechanism by which stress leads to rapid replication of WSSV in infected cells.

nisms that are used by a pathogen to thwart its host, and equally far from understanding the mechanisms by which the host attempts to thwart the invader. The present review has described just one example of how pathogenomics can be used to study pathogen-host interaction. This is an area of science that is both important and of global interest.

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