Fish & Shellfish Immunology 59 (2016) 268–275

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Shrimp STAT was hijacked by white spot syndrome virus immediate-early protein IE1 involved in modulation of viral genes

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ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form
18 October 2016
Accepted 30 October 2016
Available online 1 November 2016

Keywords:
Shrimp
WSSV
STAT
IE1
Interaction
Transcription regulation

ABSTRACT

STATs are a family of transcription factors that regulate a cascade of cellular processes including cell growth, differentiation, apoptosis and immune responses. However, they are usually targeted by viruses to assist infection. In this study, we identified that white spot syndrome virus (WSSV) immediate-early protein IE1 interacted with *Litopenaeus vannamei* STAT (LvSTAT) and thereby led to its phosphorylation activation. In addition, we demonstrated that LvSTAT could bind to the promoters of the viral immediate-early genes *wsv051* and *ie1* through STAT-binding motifs in vitro and vivo, allowing the enhancement of their promoters' activities. Moreover, IE1 could promote the transcriptional activation activity of LvSTAT to augment the transcription of *wsv051* and *ie1*. In conclusion, our findings revealed a novel linkage between WSSV IE1 and shrimp STAT, which was a clue to well understand how WSSV adopted the active strategies to modulate the shrimp signaling pathway.

1. Introduction

Signal transducers and activators of transcription (STATs) are a family of latent cytoplasmic transcription factors that comprise seven members in mammals, named STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5A and STAT5B) and STAT6 [1,2]. Upon activation by tyrosine phosphorylation, STATs can dimerize and translocate into the nucleus where they bind to their cognate DNA response elements and trigger gene transcription. In mammals, STATs are usually the common target of viruses [3]. Several viruses are known to regulate the host STATs activity to enhance replication or oncogenic potential of the viruses. For instance, Hepatitis C virus (HCV) core protein was found to directly interact with and activate the host STAT3, which led to cell growth transformation [4]. Similarly, Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded immediate-early (IE) protein ORF50 and latency-associated nuclear antigen (LANA) could also enhance the transcriptional activation activity of STAT3 by direct binding [5,6]. Previous studies have shown that white spot syndrome virus (WSSV) can induce shrimp STAT activation to facilitate the transcription of the viral gene *ie1*, suggesting that the activated STAT was conducive to WSSV infection [7,8]. A recent study further confirmed that WSSV replication was inhibited after disrupting STAT activity by gene silencing or addition with specific inhibitor of STAT [9]. Therefore, these results suggested that shrimp STAT was also targeted and exploited by WSSV. However, very little is known regarding the detailed molecular mechanism by which WSSV regulates the STAT activity in shrimp.

WSSV is a large circular double-stranded DNA (dsDNA) virus with a genome of approximately 300 kb [10,11]. It is currently the most destructive pathogen and severely threatens the worldwide shrimp farming industries [12]. Like most dsDNA viruses, the WSSV genes can be roughly divided into three categories during the viral replication cycle: IE genes, early genes and late genes [13]. In general, IE genes are often widely investigated because of their critical regulatory roles in the pathogenesis of viral infection [14–16]. Up to date, twenty-one IE genes have been identified from WSSV [17–19]. Among them, IE1, also designated as WSV069, was the focus of studies and its biological functions were frequently reported in recent years. It has been found that IE1 can act as a

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http://dx.doi.org/10.1016/j.fsi.2016.10.051
1050-4648/

transcription factor with transactivation, dimerization and DNA binding activities [20]. In addition, IE1 was demonstrated to associate with and hijack shrimp TATA box-binding protein (TBP) and thioredoxin (Trx) to improve its transcriptional activation activity [21,22]. Recently, IE1 has been also shown to bind competitively to shrimp retinoblastoma protein (Rb), which led to releasing the E2F transcription factor from Rb-E2F complex to stimulate the G_1/S transition of the cells [23]. To sum up, these data indicated that IE1 was multifaceted in functions with the abilities to regulate the transcription of targets genes, or serve as an adaptor protein involved in recruiting and manipulating host proteins to enhance viral infection.

In the present study, we further found that WSSV IE1 could physically interact with *Litopenaeus vannamei* STAT (LvSTAT), and then induced the phosphorylation activation of LvSTAT. In this way, the activated LvSTAT facilitated the transcription of the viral IE genes *wsv051* and *ie1* through their STAT binding motifs in the promoters. Hence our results indicated that IE1 could be engaged in the transcription of viral genes by influencing LvSTAT activity.

2. Materials and methods

2.1. Antibodies

Rabbit antibody against V5 tag (anti-V5) was purchased from Millipore and used for co-IP assays. Anti-V5 antibody linked with horseradish peroxidase (anti-V5-HRP) from Life Technology was used in the western blot. Mouse antibody anti-EGFP was acquired from Millipore. Mouse antibodies anti-FLAG M2 and anti-tubulin were bought from Sigma. The mouse antibody against LvSTAT was prepared in our lab by immunization with purified recombinant protein. HRP-conjuncted goat anti-mouse and anti-rabbit secondary antibodies were obtained from Thermo Scientific.

2.2. Plasmids construction

The empty vector pIZ-V5-His (Life Technology) was used to construct the plasmids for LvSTAT and IE1 expression in insect cells. For LvSTAT expression, the open reading frame (ORF) of LvSTAT were amplified by gene-specific primers and cloned into the pIZ-V5-His vector to generate the V5-tagged expression plasmid pIZ-V5-LvSTAT. In co-IP assay, the FLAG-tagged plasmid pIZ-FLAG-LvSTAT was also constructed by cloning the ORF of LvSTAT into pIZ-V5-His vector and fusing a FLAG tag to the N-terminus. For IE1 expression, the ORF of IE1 were fused with EGFP at the N-terminus by overlapping PCR. Next, the resulting ORF encoding fusion protein EGFP-IE1 were cloned into the pIZ-V5-His vector that prepared the plasmid pIZ-V5-EGFP-IE1.

For luciferase reporter plasmid constructions, the putative promoters upstream from translation start site of *wsv051* and *ie1* (447 bp in *wsv051* and 146 bp in *ie1*) were amplified by PCR and inserted into pGL3-Basic vector (Promega) to produce plasmids pGL3-*wsv051* and pGL3-*ie1*. Similarly, the *wsv051* and *ie1* promoters containing mutated STAT-binding motifs were amplified and cloned into pGL3-Basic vector to gain plasmids pGL3-*wsv051*-MUT and pGL3-*ie1*-MUT. The primers used in these constructions were listed in Table 1.

2.3. Cell culture and transient transfection

High Five cells were maintained at 27 °C in Express Five SFM medium (Life Technology). Sf9 cells were grown in Sf-900 III SFM medium (Life Technology) supplied with 5% fetal bovine serum (Gibco) at 27 °C. For transient transfection, cells were seeded overnight, and then were transfected with the required plasmids by using the Cellfectin reagent (Life Technology) according to the manufacturer's instructions.

2.4. Western blot

The whole cell lysates prepared with western and IP cell lysis buffer (Beyotime) were supplemented with 2 × SDS-PAGE sample buffer (100 mM Tris, 4% SDS, 20% glycerol, 2% β-mercaptoethanol, 0.2% bromophenol blue, pH 6.8) and boiled for 10 min. The protein samples were electrophoresed in 8%–10% SDS–PAGE gels and blotted onto PVDF membranes (GE Healthcare). The membranes were blocked for 1 h at room temperature in 5% skim milk dissolved in TBST buffer (20 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.6), and subsequently incubated with primary antibodies (1:1000) overnight at 4 °C. The membranes were then washed for three times with TBST buffer, followed by incubation for 1 h at room temperature with HRP-linked secondary antibodies (1:3000).

Table 1
Primers used for plasmid constructions.

Primer name	Sequence (5′–3′)[a,b,c]
For pIZ-V5-LvSTAT plasmid	
V5-STAT-F	CG**GGATCC**ACCATGGACATGTCGTTGTGGAACAGA
V5-STAT-R	CCG**CTCGAG**CGCTGAGGCTTCATGAAGTTGGTCTGAAG
For pIZ-FLAG-LvSTAT plasmid	
FLAG-STAT-F	CG**GGATCC**ATGGACTACAAGGACGACGATGACAAGATGTCGTTGTGGAACAGAGCACAG
FLAG-STAT-R	CCG**CTCGAG**TCACTGAGGCTTCATGAAGTTGGTCTGAAG
For pIZ-V5-EGFP-IE1 plasmid	
EGFP-F	CGG**GGTACC**ATGGTGAGCAAGGGCGAGGAG
EGFP-R	CTTGTACAGCTCGTCCATGCCGAGAG
IE1-F	CTCTCGGCATGGACGAGCTGTACAAGATGGCCTTTAATTTTGAAG
IE1-R	CCG**CTCGAG**CGTACAAAGAATCCAGAAATCTCATC
For pGL3-*ie1* and pGL3-*ie1*-MUT plasmids	
pGL3-*ie1*- F	CG**GGTACC**CCTTGTTACTCATTTATTCCTAGAAATGG
pGL3-*ie1*- MUT-F	CG**GGTACC**CCTTGTTACTCATTTA***GG***CCTAG***CC***ATGG
pGL3-*ie1*-R	CCG**CTCGAG**CTTGAGTGGAGAGAGAGAGC
For pGL3-*wsv051* and pGL3-*wsv051*-MUT plasmids	
pGL3-*wsv051*-F	CG**GGTACC**CAGTTCCAGGAAGAATGC
pGL3-*wsv051*-MUT-F	CG**GGTACC**CAG***GG***CCAGG***CC***GAATGC
pGL3-*wsv051*-R	CCG**CTCGAG**TTTCTCCCAACTTTG

[a] Nucleotides in bold were the restriction sites introduced for cloning.
[b] Nucleotides encoding FLAG tag were underlined.
[c] Nucleotides in italics and bold indicated the mutated STAT binding motif.