Biochemical and Biophysical Research Communications 415 (2011) 279–283

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Transient expression of a viral histone H4 inhibits expression of cellular and humoral immune-associated genes in *Tribolium castaneum*

Rahul Hepat, Yonggyun Kim *

Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea

ARTICLE INFO

Article history:
Received 20 September 2011
Available online 21 October 2011

Keywords:
Histone H4
Polydnavirus
Epigenetic control
Transient expression
Tribolium castaneum

ABSTRACT

A viral histone H4 is encoded in a polydnavirus called *Cotesia plutellae* bracovirus (CpBV), which is symbiotic to an endoparasitoid wasp, *C. plutellae*. Compared to general histone H4s, the viral H4 possesses an extra N-terminal tail containing 38 amino acid residues, which has been presumed to control host gene expression in an epigenetic mode. To analyze the epigenetic control activity of CpBV-H4 on expression of immune-associated genes, it was transiently expressed in larvae of *Tribolium castaneum* that had been annotated in the immune genes from a full genome sequence. Subsequent alteration of gene expression pattern was compared with that of its mutant form deleting N-terminal tail (truncated CpBV-H4). In response to bacterial challenge, *T. castaneum* induces expression of 13 antimicrobial peptide (AMP) genes. When CpBV-H4 was expressed, the larvae failed to express 12 inducible AMP genes. By contrast, when truncated CpBV-H4 was transiently expressed, all AMP genes were expressed. Hemocyte nodule formation was significantly impaired by expression of CpBV-H4, in which expressions of tyrosine hydroxylase and dihydroxyphenylalanine decarboxylase were suppressed. However, expression of truncated CpBV-H4 did not give any significant adverse effect on the cellular immunity. The immunosuppression of CpBV-H4 was further supported by its activity of enhancing bacterial pathogenicity of an entomopathogenic bacterium, *Xenorhabdus nematophila*, against larvae transiently expressing CpBV-H4. These results suggest that CpBV-H4 suppresses both humoral and cellular immune responses of *T. castaneum* by altering a normal epigenetic control of immune-associated gene expression.

1. Introduction

Polydnaviruses (PDVs) are a group of unique double-stranded DNA viruses symbiotic to some endoparasitoid wasps [1]. It comprises two genera, Ichnovirus (IV) and Bracovirus (BV), depending on their host wasp families [2]. PDV genome is located on host wasp chromosomes as a proviral form and replicated at a specific organ (ovarian calyx) during late pupal period [3,4]. Replicated PDVs form virion structure and are delivered to their specific lepidopteran host along with wasp eggs [5]. In the lepidopteran host, PDVs express their encoded genes without further replication and alter host physiological processes to be favored by wasp host development [6].

An endoparasitoid wasp, *Cotesia plutellae*, possesses a PDV called *C. plutellae* bracovirus (CpBV) and parasitizes young larvae of the diamondback moth, *Plutella xylostella* [7]. A recent deep sequencing of CpBV genome provides 157 putative genes containing five typical PDV gene families [8]. With the help of CpBV, parasitization of *C. plutellae* induces significant immunosuppression of parasitized larvae and extends larval period, which finally inhibits metamorphosis to pupal stage [9,10]. To manipulate host physiological processes, CpBV expresses its own genes, such as CpBV-PTPs [11], CpBV-ELPs [10], CpBV-IkBs [12] and CpBV-HTIFs [13].

A viral histone H4 is identified in CpBV genome [14]. It exhibits a high sequence homology with histone H4 of *P. xylostella* except N-terminal 38 residues [15]. A similar viral histone H4s are reported in other *Cotesia*-associated PDVs, such as *C. congregata* bracovirus (CcBV) and *C. glomerata* bracovirus (CgBV) [16–18]. All viral histone H4s contain N-terminal tail, which has been suggested to play a crucial role in manipulating host gene expression in an epigenetic mode.

This study used a model insect, *Tribolium castaneum* that was identified in its full genome and subsequently annotated in immune-associated genes [19,20]. The epigenetic control function of the N-terminal tail of CpBV-H4 was proved by comparing its control effect on expression of immune-associated genes of *T. castaneum* with that of truncated CpBV-H4 constructed by deleting N-terminal tail. Furthermore, this study demonstrated the immunosuppression

Abbreviations: CpBV, *Cotesia plutellae* bracovirus; PDV, polydnavirus; CpBV-H4, *Cotesia plutellae* bracovirus histone H4; AMP, antimicrobial peptide gene; DOPA, 3,4-dihydroxyphenylalanine.

* Corresponding author. Fax: +82 54 823 1628.
E-mail address: hosanna@andong.ac.kr (Y. Kim).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.10.040

induced by expression of CpBV-H4 by enhanced pathogenicity of an entomopathogenic bacterium, *Xenorhabdus nematophila*, against *T. castaneum*.

2. Materials and methods

2.1. Insect rearing and bacteria culture

T. castaneum was reared in a dry and dark condition (a relative humidity 60 ± 5%) at room temperature (25 ± 1 °C) with wheat flour (Pareve, USA). Fully grown late instar larvae (⩾5 mm) were used in this study. Both Gram-negative bacteria, *Escherichia coli* (non-entomopathogenic) and *X. nematophila* K1 (entomopathogenic), were cultured in Luria–Bertani (LB) broth at 28 °C with shaking at 200 rpm.

2.2. cDNA construction

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and followed by reverse transcription using RT-Premix (Bioneer, Daejon, Korea) with an oligo dT (5′-CCAGTGAGCAG AGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTT-3′) and subsequent RNase H treatment. The synthesized cDNA was used as a template for PCR amplification. Control PCR was performed with RNA extract as a template to check absence of DNA contamination.

2.3. Construction of viral recombinants

A full open reading frame of CpBV-H4 was obtained from parasitized *P. xylostella* using two gene-specific primers: forward 5′-GGATCCATGGCTGATCATCCTAAAGG-3′ and reverse 5′-GAATTCTCA ACCTCCATAACCATAGATC-3′ (BamHI and EcoRI sites are underlined). Truncated CpBV-H4 (without N-terminal 38 amino acids) was produced with two gene-specific primers: forward 5′-GGATCCATGGGAAGAGGATTGGGCAA-3′ and reverse 5′-GAATTCTCAACC TCCATAACCATAGATC-3′ (BamHI and EcoRI sites are underlined). PCR product cloned into pIB eukaryotic expression vector (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions.

2.4. Transient expression of CpBV-H4 and truncated CpBV-H4 in T. castaneum

Each recombinant pIB vector was mixed with Metafectene PRO transfection reagent (Biontex, Planegg, Germany) according to manufacturer's instruction. Briefly, 0.5 μg of recombinants were mixed with 3 μl of Metafectene reagent and incubated for 20 min at room temperature to allow DNA–lipid complexes to be formed before injection into hemocoel of late instar larvae of *T. castaneum*. Glass capillary injection needles were made using a Micropipitte puller PN30 (Narishige, Tokyo, Japan). A total 60 nl was injected into larval hemocoel at a rate of 10 nl/s using Microsyringe pump controller (World Precision Instruments, Sarasota, FL, USA) under a microscope (Olympus S730, Tokyo, Japan).

2.5. Hemocyte nodulation assay

Nodulation assay was performed by injecting 5×10^4 cells of *E. coli* at 48 h after infection of recombinant CpBV-H4 or truncated CpBV-H4 vector. A total 60 nl was injected into hemocoel of late instar *T. castaneum* as described above. After 8 h incubation at room temperature, melanized black nodules were counted externally on its ventral side through transparent body cuticle under stereomicroscope (Olympus SZX9) at 50× magnification.

2.6. Immune-associated genes and RT-PCR

A previous study [19] annotated genes associated with immune responses of *T. castaneum*, in which 13 genes of putative AMPs (cecropin 2, GLEAN00499; cecropin 3, GLEAN00500; attacin 1, GLEAN07737; attacin 2, GLEAN07738; attacin 3, GLEAN07739; lysozyme 1,GLEAN10349; lysozyme 2, GLEAN1035; lysozyme 3, GLEAN10351; lysozyme 4, GLEAN10352; defensin 1, GLEAN0625; defensin 2, GLEAN10517; defensin 3, GLEAN12469, coleoptericin 1, GLEAN05093), tyrosine hydroxylase (EF592178), and dihydroxyphenylalanine (DOPA) decarboxylase (NM-001102586) were retrieved from the beetlebase (http://www.beetlebase.org).cDNA was prepared as described above and used for PCR amplification with specific primers (Supplementary Table 1) for the immune-associated genes with 35 cycles under the conditions of 1 min at 94 °C for denaturation, 30 s for annealing with specific temperature for each gene, and 1 min at 72 °C for extension.

2.7. Statistical analysis

All studies were performed in three independent replicates and plotted by mean ± standard deviation using Sigma plot. Means were compared by a least squared difference (LSD) test of one way ANOVA using PROC GLM of SAS program and discriminated at Type I error = 0.05.

3. Results

3.1. Transient expression of CpBV-H4 or its truncated form in T. castaneum

To determine physiological roles of CpBV genes, each specific viral gene has been transiently expressed in nonparasitized larvae of *Plutella xylostella* using an eukaryotic expression vector, pIB [10,11,21]. This study applied this transient expression technique to larvae of *T. castaneum*. Two pIB recombinant constructs expressing CpBV-H4 or truncated CpBV-H4 were prepared by gene-specific primers and subsequent PCR cloning (Fig. 1A). When these recombinant pIB vectors were injected to larvae of *T. castaneum*, insert genes were expressed at 12 h post injection (PI) and maintained their expression until 96 h PI (Fig. 1B). Two construct expressions were easily discriminated with their specific PCR primers, in which truncated CpBV-H4 expression was not detected by N-terminal tail forward primer (Fig. 1C). Thus, the expression of truncated CpBV-H4 could be specifically analyzed.

3.2. CpBV-H4 inhibits inducible expression of AMP genes

Based on transient expression of CpBV-H4 and its truncated form, we analyzed their effects on expression of AMP genes in response to bacterial challenge (Fig. 2). All 13 AMP genes showed little expression at no immune challenge; however, injection of *E. coli* to hemocoel showed marked increases in their expressions. Transient expression of CpBV-H4 or its truncated form did not change in basal expression levels of these AMP genes at no immune challenge. However, CpBV-H4 inhibited the inducible expressions of these AMP genes except lysozyme 3. Interestingly, three genes (attacin 2, cecropin 2 and lysozyme 4) were inhibited even in their basal expression levels upon the bacterial challenge. However, the truncated CpBV-H4 lost all inhibitory activities and was not different with control treatment.

3.3. CpBV-H4 inhibits cellular immune response of T. castaneum

Larvae of *T. castaneum* showed hemocyte nodule formation in response to bacterial challenge (Fig. 3A). However, the transient