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IkB genes encoded in *Cotesia plutellae* bracovirus suppress an antiviral response and enhance baculovirus pathogenicity against the diamondback moth, *Plutella xylostella*

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ABSTRACT

An endoparasitoid wasp, *Cotesia plutellae*, parasitizes larvae of the diamondback moth, *Plutella xylostella*, with its symbiotic polydnavirus, *C. plutellae* bracovirus (CpBV). This study analyzed the role of Inhibitor-kB (IkB)-like genes encoded in CpBV in suppressing host antiviral response. Identified eight CpBV-IkBs are scattered on different viral genome segments and showed high homologies with other bracoviral IkBs in their amino acid sequences. Compared to an insect ortholog (e.g., Cactus of *Drosophila melanogaster*), they possessed a shorter ankyrin repeat domain without any regulatory domains. The eight CpBV-IkBs are, however, different in their promoter components and expression patterns in the parasitized host. To test their inhibitory activity on host antiviral response, a midgut response of *P. xylostella* against baculovirus infection was used as a model reaction. When the larvae were orally fed the virus, they exhibited melanotic responses of midgut epithelium, which increased with baculovirus dose and incubation time. Parasitized larvae exhibited a significant reduction in the midgut melanotic response, compared to nonparasitized larvae. Micro-injection of each of the four CpBV genome segments containing CpBV-IkBs into the hemocoel of nonparasitized larvae showed the gene expressions of the encoded IkBs and suppressed the midgut melanotic response in response to the baculovirus treatment. When nonparasitized larvae were orally administered with a recombinant baculovirus containing CpBV-IkB, they showed a significant reduction in midgut melanotic response and an enhanced susceptibility to the baculovirus infectivity.

1. Introduction

An endoparasitoid wasp, *Cotesia plutellae* (=*vestalis*), carries a symbiotic polydnavirus, *C. plutellae* bracovirus (CpBV) (Bae and Kim, 2004). When *C. plutellae* parasitizes the diamondback moth, *Plutella xylostella*, it delivers the viral particles along with the wasp egg into the host hemocoel (Basio and Kim, 2006). Parasitized *P. xylostella* exhibits immunosuppression and a delay of larval development (Lee et al., 2005; Ibrahim et al., 2007).

Polydnavirus is an insect DNA virus, which is characterized by its segmented genome located on the host wasp chromosome as a provirus (Krell et al., 1982). Viral replication occurs only in female reproductive organs during pharate adult stage in response to ecdysteroid rise (Webb and Summers, 1992). Its total genome size at episomal viral form ranges from 160 kb to 545 kb, depending on the viral species (Espagne et al., 2004; Webb et al., 2006). Polydnaviral genes predicted from the genome sequences can be grouped into protein tyrosine phosphatase (PTP), Inhibitor-kB (IkB)-like, cysteine-rich gene, vinnexin, rep gene families as well as numerous hypothetical genes (Kroemer and Webb, 2004).

Over 70% of the CpBV genome has been sequenced and allows us to predict different polydnaviral gene families (Choi et al., 2005). The largest gene family is CpBV-PTP which contains at least 39 predicted genes encoded in different CpBV segments (Ibrahim et al., 2007). Expression of CpBV-PTP can modify cellular phosphatase activity of hemocytes and results in the suppression of cellular immune responses (Ibrahim and Kim, 2008). The second largest family of CpBV genes is IkB-like gene family which includes at least eight putative genes (Kim et al., 2006; Shi et al., 2008; this study). The physiological role(s) of these genes in the parasitism was not known in this species.

IkB-like genes have been reported in both polydnaviral groups of ichnovirus and bracovirus (Webb et al., 2006), indicating a common parasitic factor probably for their viral survival. *Drosophila melanogaster* has two independent humoral immune signaling pathways, both of which lead to the activation of NF-kB transcription factors. One pathway responds primarily to fungal and Gram-positive bacterial infection, while the other responds to lipopolysaccharide treatment or infection by Gram-negative bacteria (Lemaitre et al., 1996, 1997). In normal cells, NF-kB/Rel fam-

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ily proteins exist as hetero- or homo-dimeric proteins that are sequestered in the cytoplasm by virtue of their association with a member of the IkB family proteins. Some extracellular signals can trigger distinct signal transduction pathways, each of which culminates in the destruction of IkB proteins. These signal transduction pathways lead to the activation of the IkB kinase (Karin and Ben-Neriah, 2000), and the subsequent phosphorylation of serine residues in the N-terminal regulatory domain (Ghosh et al., 1998). Phosphorylated IkB proteins are followed by the ubiquitination and degradation by proteasome (Karin and Ben-Neriah, 2000). The degradation of IkB unmasks the nuclear localization signal of the NF-kB/Rel family protein, leading to its nuclear translocation and binding to enhancers or promoters of related immune genes. Indeed, IkB-like gene of *Microplitis demolitor* bracovirus can bind NF-kBs of *D. melanogaster* and inhibit their nuclear translocation, resulting in the suppression of antimicrobial peptide production (Thoetkiattikal et al., 2005). IkB-like genes of *Chelonus inanitus* bracovirus and *Campoletis sonorensis* ichnovirus enhance baculovirus oral infectivity against a virus resistant *Spodoptera littoralis* (Rivkin et al., 2006). However, the functional link between the baculoviral infectivity and the immunosuppressive activity of IkB-like genes remain unknown. In *D. melanogaster*, Toll pathway is required to express antiviral response (Zambon et al., 2005). These led us to pose a hypothesis that CpBV-IkBs may suppress host antiviral response(s), at which baculovirus pathogenicity would be enhanced. To test this hypothesis, this study analyzed host midgut responses following oral administration of a recombinant *Autographa californica* multiple nucleopolyhedrosis virus expressing enhanced green fluorescence protein (AcMNPV-EGFP). The study also analyzed how the parasitization by *C. plutellae* inhibited the responsiveness of *P. xylostella* to the baculovirus. Any contribution of CpBV-IkBs to the antiviral response was investigated by transient expression of the viral genes. Finally, this study demonstrated that a recombinant AcMNPV expressing a CpBV-IkB enhanced the viral pathogenicity against *P. xylostella*.

2. Materials and methods

2.1. Insect rearing and parasitization

P. xylostella larvae were reared on cabbage leaves at a temperature of 25 ± 1 °C and 16:8 h (L:D) photoperiod. Adults were fed 10% sucrose. Late second instar larvae (4 days after oviposition at 25 °C) were parasitized by *C. plutellae* at ≈1:2 (wasp:host) ratio for 24 h. Then, the parasitized larvae were fed and reared on cabbage leaves at the rearing environment. Adults that emerged from the cocoons (11 days after parasitization at 25 °C) were collected and allowed to mate for 24 h before use for parasitization.

2.2. Analysis of CpBV-IkBs sequence

CpBV-IkB sequences were obtained from NCBI-GenBank with accession numbers (CpBV-IkB1; AAZ04265, CpBV-IkB2; AAZ04266, CpBV-IkB3; AAZ04279, CpBV-IkB4; AAZ04280, CpBV-IkB5; AAZ04281, CpBV-IkB6; AAZ04283, CpBV-IkB7; AAZ04284, CpBV-IkB8; ABK63306). The derived amino acid sequences of CpBV-IkBs using LASERGENE software (DNASTAR, Madison, WI) were aligned to other 17 IkBs derived from other polydnaviruses and mammals by using CLUSTAL_W. Putative posttranscriptional modification and functional domains were analyzed by a protein motif search program (http://www.ebi.ac.uk). Putative promoters (1 kb upstream from ATG start codon) of CpBV-IkBs were analyzed by TESS (http://www.cbil.upenn.edu) and geWorkbench version 1.6.

2.3. RT-PCR

Total RNA of *P. xylostella* parasitized or nonparasitized by *C. plutellae* was isolated using Trizol (Invitrogen, Carlsbad, CA, USA). To analyze tissue-specific expression, hemocytes, gut, and fat body were isolated from the 4th instar larvae parasitized by *C. plutellae* and used for RNA extractions. The total RNAs were treated with DNase I for 1 h prior to cDNA synthesis to remove contaminating viral DNA. No DNA contamination was confirmed by PCRs using the RNA templates. First strand cDNA was synthesized with 4 μg of the DNase-treated RNA using RT-Premix oligo-dT (Intron, Seoul, Korea). Gene specific primers of CpBV-IkBs were designed (Table 1) and used to amplify the cDNAs. PCR program included a pre-denaturation (95 °C, 2 min), followed by 35 amplification cycles (denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, chain extension at 72 °C for 1 min) and final extension at 72 °C for 10 min.

2.4. Cloning of CpBV segments

CpBV genomic DNA was extracted by the method described by Bae and Kim (2004). The genome segments were cloned with a transposon of pPCS-S described by Choi et al. (2005). The resulting CpBV genome segments were individually purified by a plasmid DNA miniprep kit (Exprep™ plasmid SV, GeneAll, Seoul, Korea). CpBV genome segments (NCBI accession number) used in this study included S2 (DQ075354), S8 (DQ075358), S9 (DQ075359), and S16 (EF067321).

2.5. Construction of recombinant AcMNPVs

pGOZA baculovirus construction method (Je et al., 2001) was used to prepare the recombinant baculovirus using pBacpak8 vector (Clontech, Paolo Alto, CA, USA). It has a polyhedrin gene promoter region to effectively express the inserted gene. Construction of AcMNPV-EGFP or IkB3 (AcMNPV-IkB3) from the recombinant baculovirus in Sf9 cells was performed according to manufacturer's instruction (Invitrogen). Briefly, the inserted DNA was amplified and cloned into the pCR2.1 vector (Invitrogen). After restriction digestion, the directional cloning was performed to rejoin the inserts and pBacpak8 with T4 ligase (Intron).

2.6. Analysis of antiviral response

After oral administration of AcMNPV-EGFP to the third instar larvae, the gut was isolated at different incubation periods and washed with phosphate-buffer saline (PBS: 0.14 M NaCl, 2.5 mM

Table 1
Gene-specific primers used to clone eight different IkB-like genes encoded in *C. plutellae* bracovirus (CpBV).

Genes	Direction	Sequence
CpBV-IkB1	Forward	5′-ACA AGG GAC ACA CAG CAT GGC ACA TGG C-3′
	Reverse	5′-ACT TGG TTG AAA TTC GTT GCG C-3′
CpBV-IkB2	Forward	5′-ATG GAG TCC GAC ATG ATT CTA C-3′
	Reverse	5′-CTC ATT ACG ATT TTT CAA CTC CAA AGG-3′
CpBV-IkB3	Forward	5′-ATG AAT AAG TCA AAT ATA GTA TTT ACC GG-3′
	Reverse	5′-CAA CTG AAG CCT TTG AGT CTT GCA C-3′
CpBV-IkB4	Forward	5′-ATG GAA GAA AAT GGA GCT GCT AAC G-3′
	Reverse	5′-CCT CGG AGT CAA CGC AAG ACT TTC-3′
CpBV-IkB5/6	Forward	5′-ATG CTA AAT GCC AAC AGT GTA CAC-3′
	Reverse	5′-CAA CTG AAG CCT TTG AGT CTT GCA C-3′
CpBV-IkB7	Forward	5′-CCT TCC GAC GAG TCT GAC AC-3′
	Reverse	5′-CGT CTT CTT GCT GAG ACG CA-3′
CpBV-IkB8	Forward	5′-ATG GAT TGG TCC GGC AAT TTC GTG-3′
	Reverse	5′-GAT TTT ATT ATC AAC TTC TAC ATC AGA C-3′