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Cotesia plutellae Bracovirus Suppresses Expression of an Antimicrobial Peptide, Cecropin, in the Diamondback Moth, *Plutella xylostella*, Challenged by Bacteria

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An endoparasitoid wasp, *Cotesia plutellae*, induces significant immunosuppression of host insect, *Plutella xylostella*. This study was focused on suppression in humoral immune response of *P. xylostella* parasitized by *C. plutellae*. An EST database of *P. xylostella* provided a putative cecropin gene (PxCec) which is 627 bp long and encodes 66 amino acids. A signal peptide (22 amino acids) is predicted and two putative O-glycosylation sites in threonine are located at positions 58 and 64. Without bacterial infection, PxCec was expressed in pupa and adult stages but not in the egg and larval stages. Upon bacterial challenge, however, the larvae expressed PxCec as early as 3 h post infection (PI) and maintained high expression levels at 12-24 h PI. By 48 h PI, its expression noticeably diminished. All tested tissues of bacteria-infected *P. xylostella* showed PxCec expression. However, other microbes, such as virus and fungus, did not induce the PxCec expression. Parasitization by *C. plutellae* suppressed the expression of PxCec in response to bacterial challenge. Among the parasitic factors of *C. plutellae*, its symbiotic virus (*C. plutellae* bracovirus: CpBV) alone was able to inhibit the expression of PxCec of *P. xylostella* challenged by bacteria. These results indicate that PxCec expression is regulated by both immune and developmental processes in *P. xylostella*. The parasitization by *C. plutellae* inhibited the expression of PxCec by the wasp's symbiotic virus.

***Keywords*:** polydnavirus, cecropin, *P. xylostella*, *C. plutellae*, expression, immunosuppression

Upon microbial infection, insects activate their innate immune system consisting of cellular and humoral components (Beckage, 2008). The cellular response is hemocyte-mediated and includes encapsulation, phagocytosis, and nodule formation, while the humoral response comprises a number of effector molecules, such as phenoloxidase and inducible antimicrobial peptides (Vizioli and Salzet, 2002). Among these inducible peptides are the cecropins which are first reported in the cecropia moth, *Hyalophora cecropia* (Hultmark *et al*., 1980). The cecropins from *Hyalophora* are 35-37 amino acids long and clustered into a family of strongly amphiphatic peptides (Steiner *et al*., 1988). Cecropins have also been isolated from nonlepidopterans including *Drosophila* (Kylsten *et al*., 1990). Mature cecropins are composed of highly basic amino acid residues that can fold into two amphiphatic α-helices and integrate into the acidic cell membranes of bacteria to lead to a disruption of the cell membrane (Steiner *et al*., 1988).

Host immunosuppression is a common parasitic strategy of various polydnaviruses (PDVs) (Webb and Strand, 2005). PDVs are a group of insect DNA virus and are symbiotic to some ichneumonid endoparasitic wasps (Stoltz *et al*., 1984). PDVs are further subdivided into bracovirus (BV) and ichnovirus (IV) depending on host wasp families and viral morphology (Webb *et al*., 2000). The viral DNAs are integrated into host wasp chromosomes to establish unique proviral forms and are replicated only in female reproductive organ to produce episomal viral particles (Krell *et al*., 1982). When the female wasps parasitize lepidopteran hosts, the polydnaviral particles enter the hemocoel along with the wasp eggs and infect target tissues including hemocytes and fat body (Barat-Houari *et al*., 2006). Even though no further replication occurs in the parasitized host, the virus expresses their genes in the target tissues to alter host development and immune responses (Theilmann and Summers, 1986). The viral genes are classified into different families depending on conserved domain structures, such as Cys-motif, Vankyrin, Rep, PTP, EGF, EP1, and Vinnexin (Kroemer and Webb, 2004). Some of the gene families have been known in their parasitic functions. For example, two Cys-motif genes, VHv1.4 and VHv1.1, are known to suppress hemocyte behavior by altering cytoskeletal rearrangement (Cui *et al*., 1997).

An endoparasitoid wasp, *Cotesia plutellae*, parasitizes the diamondback moth, *Plutella xylostella*, which undergoes developmental arrest and immunosuppression (Bae and Kim, 2004). The wasp contains a symbiotic PDV called *C. plutellae* bracovirus (CpBV), which has been known to be a main parasitic factor because of a number of genes encoded in the viral genome (Kim *et al*., 2007). CpBV15β inhibits hemocyte-spreading behavior by inhibiting protein synthesis (Madanagopal and Kim, 2007). CpBV-PTPs suppress hemocyte encapsulation behavior by altering intracellular phosphorylation levels (Ibrahim and Kim, 2006). CpBV-lectin prevents recognition of hemocytes by masking wasp eggs (Nalini *et al*., 2008). CpBV-H4 also inhibits hemocyte-spreading behavior by its epigenetic control activity (Wael and Kim, 2009).

Several parasitic factors encoded in CpBV genome, such as

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CpBV-IkB, have been suspected to suppress host humoral immune response (Bae and Kim, 2009). A current EST database introduced a cecropin gene in *P. xylostella* (PxCec) but its expression was not confirmed in *P. xylostella*. This study analyzed the expressions of PxCec during different developmental stages in naïve *P. xylostella*. It also analyzed its inducible expression pattern to different microbes. Finally, this study showed that the expression of PxCec was suppressed by parasitization of *C. plutellae* due to its symbiotic virus, *C. plutellae* bracovirus (CpBV).

Materials and Methods

Insect rearing and parasitization

Nonparasitized (NP) larvae of *P. xylostella* were reared under 25±1°C and 16:8 h (L:D) photoperiod with cabbage leaves. Adults were fed 10% sucrose. Late second instar larvae were parasitized by *C. plutellae* at 1:2 (wasp:host) density for 24 h under the rearing conditions. The parasitized (P) larvae were fed and reared on cabbage leaves at the rearing environment. After emergence, adult wasps were allowed to mate for 24 h and then used for parasitization.

Antibacterial activity

Antimicrobial activity of the plasma of *P. xylostella* was analyzed by inhibition zone assay. Fourth instar NP and P larvae were injected with *Escherichia coli* Top10 (5×10^4 cells, Invitrogen, USA) using a nanoliter injector (Nanojet II, WPI, Inc., USA) equipped with a micro-syringe pump controller (WPI) under a microscope (Olympus S730, Japan). Capillary tubes for micro-injection were prepared using a Microelectrode Puller (PN-30, Narishige Co. Ltd., Japan) and were half-filled with mineral oil before taking up samples. A total of 500 nl was injected into larval hemocoel at 10 nl/sec. Hemolymph was collected in phenylthiocarbamide (Sigma-Aldrich Korea, Korea) at 6 h post-infection (PI) and centrifuged at 3,000 rpm for 5 min to get the supernatant plasma. Plasma samples were diluted with anticoagulant buffer, which was prepared fresh by dissolving 4 mg L-cysteine hydrochloride (Sigma, USA) in 5 ml Tris-buffered saline (50 mM Tris/HCl; pH 7.5, 100 mM glucose, 5 mM KCl, 2.5 mM $MgCl_2$, and 50 mM NaCl). Each experiment was independently replicated thrice.

RNA extraction and reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNAs were extracted from NP and P larvae of *P. xylostella* using Trizol reagent (MRC, USA) and precipitated with isopropanol. RNA pellet was washed with 70% ethanol and finally resuspended in DEPC-treated water. Total RNAs from *P. xylostella* challenged with a Gram positive bacterium (*Bacillus subtilis*), a fungus (*Jeffersonia dubia*) or a baculovirus (*Autographa californica* nucleopolyhedrovirus) were also extracted. Total RNA (1 μg) was reverse-transcribed with RT-PCR premix (Intron, Korea). The resulting cDNA was used as template to amplify a cecropin gene using primers (5′-ATG AAA CTG TCA AAT ATT TTC-3′, 5′-CCC AGT AGG TCT GGC TA-3′). The PCR reaction was performed in a total volume of 20 μl and was run for 30 cycles of denaturation at 94°C for 20 sec, annealing at 50°C for 20 sec and extension at 72°C for 1 min, and followed by final extension at 72°C for 5 min.

Sequence analysis

ORF sequence of cecropin was predicted using the DNA star program (Version 5.02, DNAstar Inc, USA). Homologous genes were identified through NCBI BLAST search. Alignment with different cecropin genes was performed using DNA star program's CLUSTAL W method with the following parameters: gap penalty, 10; gap length penalty, 0.20; delay divergent seq, 30%; DNA transition width, 0.50. Post-translational modifications on cecropin were predicted using SignalP3.0 and NetOGlyc3.1 programs (http://us.expasy.org/). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tanura *et al*., 2007) and Mobyle@Pasteur Phylip drawtree program version 3.5c (Felsenstein, 1989).

PDV extraction and transient expression

Reproductive tracts of 30 female *C. plutellae* were removed and placed in 1×50 mM phosphate buffered saline (PBS), pH 7.0. Venom glands were carefully removed during dissection. The ovarian calyx was punctured and the PDV suspension was collected into a microcentrifuge tube on ice. The suspension was centrifuged for 10 min at 11,000 rpm at 4°C and washed with PBS twice. The pellet was resuspended in 15 μl of PBS. The extracted CpBV was used immediately for microinjection. One microliter of CpBV was injected into NP larvae using the glass capillary. Microinjection was performed under a microscope (Olympus S730, Japan). The total RNA extracted from these treated insects were used to analyze transient expression of PxCec and a CpBV-encoded gene, CpBV-H4 (FP: 5′-CGG GAT CCA TGG CTG ATC ATC CTA AAG G-3′; RP: 5′-CGG AAT TCA CCT CCA TAA CCA TAG ATC-3′). CpBV-IkB4 (FP: 5′-ATG GAA GAA AAT GGA GCT GCT AAC G-3′; RP: 5′-CCT CGG AGT CAA CGC AAG AC-3′). β-Actin was used as a control (FP: 5′-TGG CAC CAC ACC TTC TAC-3′, RP: 5′-CAT GAT CTG GGG TCA TCT TCT-3′).

Results

Suppression in antibacterial activity of *P. xylostella* parasitized by *C. plutellae*

The influence of *C. plutellae* parasitization on humoral immune response of *P. xylostella* was analyzed by bacterial inhibition zone assay (Fig. 1). The plasma obtained from NP larvae that had been infected with bacteria for 6 h possessed significant antibacterial activity because it inhibited bacterial growth on the culture plate, which was easily recognized by a clear zone around the treated paper disc (Fig. 1A). The undiluted NP plasma showed inhibition zone comparable of that of 250 ppm of ampicillin (Fig. 1B). Serial dilutions of the NP plasma showed the corresponding decreases in the size of the inhibition zone (data not shown). However, the undiluted plasma obtained from the P larvae treated with an identical immune challenge did not form any clear zone.

A cecropin gene encoded in *P. xylostella* genome and phylogenetic analysis

Using a constructed expressed sequence tag (EST) clone collection and cDNA microarray profiling analyses, several immune-inducible genes were identified in *P. xylostella* (Eum *et al*., 2007) including a putative cecropin (PxCec), which is 627 bp long and encodes 66 amino acid sequence (Fig. 2A). A signal peptide (22 amino acids) is predicted and two putative O-glycosylation sites in threonine are located at positions 58 and 64. There is a polyadenylation signal sequence of AATAAA at 3′ untranslated region. PxCec in genomic DNA of *P. xylostella* contains an intron sized 226 bp long in open reading frame (ORF) region (Fig. 2B). Phylogenetic and molecular

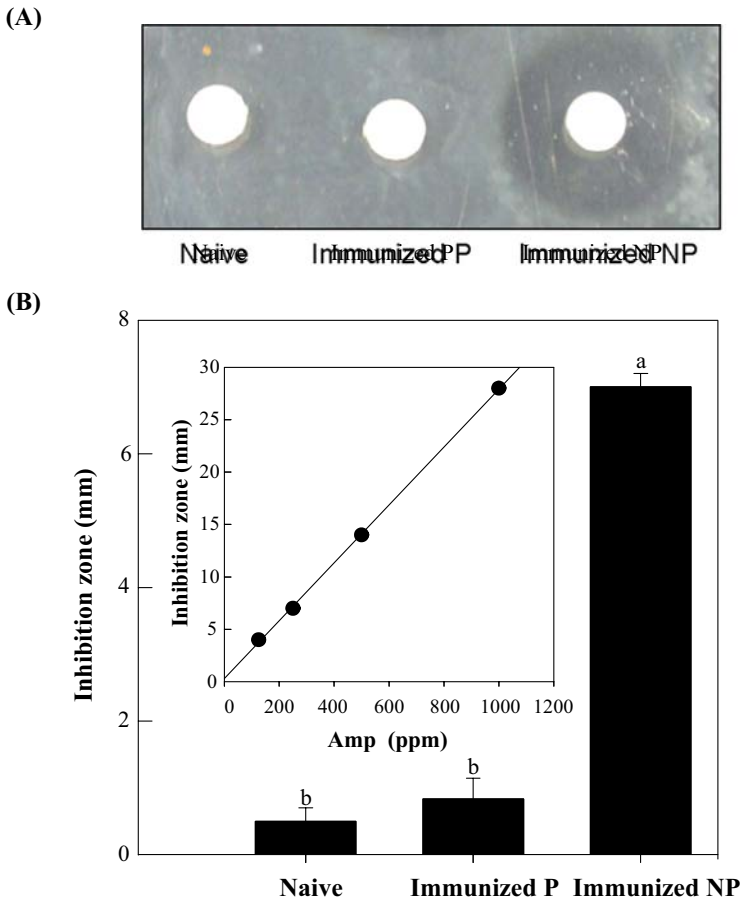

Fig. 1. Suppressed antibacterial activity of *P. xylostella* parasitized (P) by *C. plutellae.* (A) Clear zone assay of nonparasitized (NP) larvae challenged by *E. coli* or naïve larvae. Fourth instar NP and P larvae were injected with *E. coli* Top10 ($5x10^4$ cells) 6 h before obtaining the plasmas. Each 10 µl of plasma from the treated larvae were applied on a sterilized paper disk. (B) Measurements of the clear zones among treatments. Different concentrations of ampicillin (amp) were used as reference (inset). Each treatment was replicated thrice. Different letters above the standard deviation bars represent significant differences among means at type I error=0.05 (LSD test).

evolutionary analyses suggest its close homology to type A cecropins of other lepidopteran insects (Fig. 2C).

Expression of PxCec associated with development and immunity

We checked expression of PxCec during different developmental stages of *P. xylostella* (Fig. 3). Without bacterial challenge, its expression was detected in prepupal cocooning, pupal, and adult stages (Fig. 3A). It was not expressed in naive larvae but expressed by bacterial infection (Fig. 3B). But the expression of PxCec was not induced by immune challenge with virus or fungi. PxCec expression in response to bacterial challenge was detected as early as 3 h PI and was mostly abundant from 12-24 h PI (Fig. 3C). The PxCec expression in response to bacterial challenge was induced in all three tissues: fat body, gut, and hemocyte (Fig. 3D).

CpBV inhibits expression of PxCec in response to bacterial challenge

To explain the suppression of antibacterial activity in P larvae shown in Fig. 1, we analyze the effect of *C. plutellae* parasitization on the expression of PxCec (Fig. 4). The parasitization inhibited the expression of PxCec (Fig. 4A). To determine the effect of CpBV on the inhibition of the PxCec expression, the fresh virus particles were collected from ovarian calyx of the female wasps and injected to NP larvae. After 24 h, the treated larvae showed expressions of CpBV genes, such as CpBV-H4 and CpBV-IkB4 (Fig. 4B). However, these treated larvae failed to express PxCec in response to bacterial challenge. Furthermore, the inhibitory activity of CpBV against PxCec expression was dependent on the viral dose.

Discussion

This study reports a cecropin gene encoded in *P. xylostella* genome. Its identity is supported by a phylogenetic sequence analysis, where it is classified into type A cecropin. It contains an intron and two exons, which encodes 66 amino acids. Most premature cecropins have 62-64 amino acid residues (van Hofsten *et al*., 1985; Gudmundsson *et al*., 1991). This precursor usually undergoes post-translational modifications. First, the N-terminal 22 amino acid residues may be cleaved by signal peptidase. Then the following two residues, Ala-Pro, may be deleted by aminodipeptidase (Boman *et al*., 1989).

(A)

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ggcacgagga aataatcgag gaacaaaaga ttccaatttc aaaaatgaaa ctgtcaaata    60
                                                 M  K  L  S  N       5
ttttcttttt cgtcttcatg gcgtttttcg cagtggccag tgtgtcggcc gcgcccaggt   120
I  F  F  F   V  F  M   A  F  F  A  V  A  S   V  S  A  A  P  R       25
ggaagccgtt taaaaaattg gaaaaagttg ggcgcaacat ccgtaacggt atcatcaggt   180
W  K  P  F   K  K  L   E  K  V  G  R  N  I   R  N  G  I  I  R       45
ataacggtcc ggcggtcgcc gtcatcggac aagccacttc tatagccaga cctactggga   240
Y  N  G  P   A  V  A   V  I  G  Q  A  T  S   I  A  R  P  T  G       65
aatgatagac ataaattaga tctaggaact tgcatttata atagctctaa tttccacgcc   300
K                                                                   66
acgcaacact tatgatttaa tccttttacc ggcataggca gtgactttaa atattgatac   360
catatttcat tttttggtcc gttaacgggt taaataatgt acacattcaa aagaatactc   420
attcttttat acagtaatta agtgaaaaag ccataattag taaacaaata gaattgtcta   480
tttatagtga tttttacatt gtaaataatt taatgataag tagatctgat attttcttag   540
acaagtaaaa aataatgtat taatagtata gttactttca ttattgtaat tattttaaaa   600
taaaatattt tagtacaata aaaaaaa                                      627
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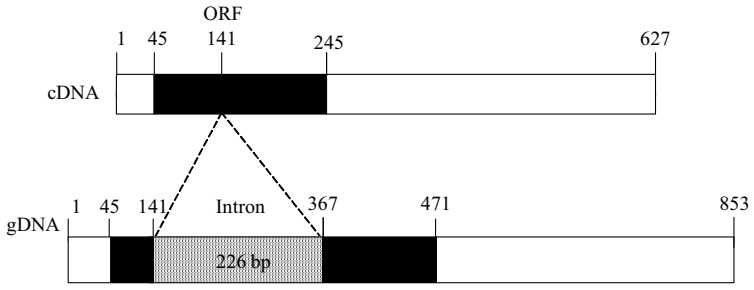
(B)



(C)

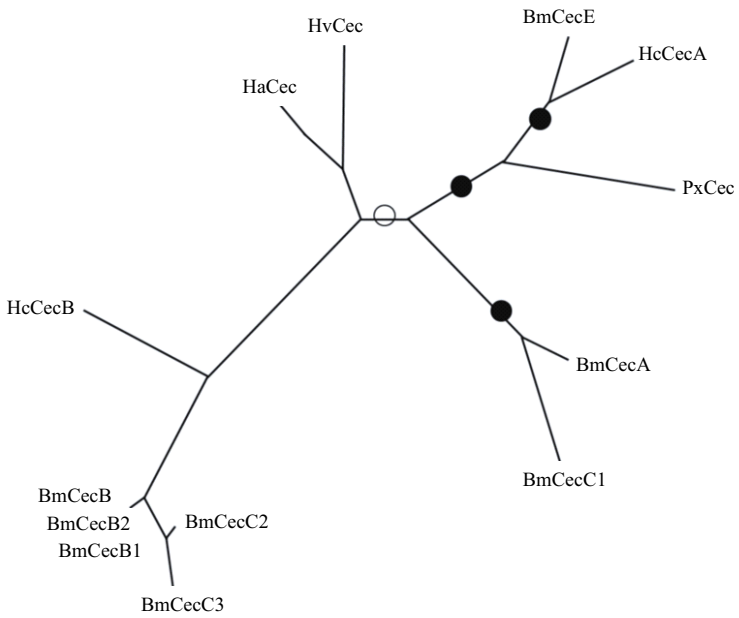


Fig. 2. Nucleotide and amino acid sequences of a cecropin gene of *P. xylostella* (PxCec). (A) PxCec cDNA is 627 bp long with an open reading frame (ORF, 201 bp) encoding 44 amino acids after putative signal peptide (bold letters) is removed. Predicted O-glycosylation sites in threonine at 58 and 64 are boxed. Start and stop codons are underlined. Positively charged amino acids, Arg and Lys, which are known to bind to the negatively charged bacterial cell membrane, are shaded. Polyadenylation signal sequence is double underlined. (B) Schematic representation showing the presence of intron located in the PxCec ORF. The nucleotide sequence containing exons, intron, and 5′, 3′ untranslated regions was deposited to GenBank with accession number of GQ479802. (C) Distance tree showing evolutionary relationships among lepidopteran cecropins. Bootstrap values were generally low (<50%) with the value of 50-80% represented by an open circle and higher than 80% is marked with filled circles. Accession numbers are: BmCecB1 (*B. mori*, NM_001043927), BmCecB2 (*B. mori*, NM_001043995), BmCecB (*B. mori*, EU047749), BmCecC3 (*B. mori*, NM_001043567), BMCecC2 (*B. mori*, NM_001043566), HcCecB (*H. cecropia*, P01508), HvCec (*H. virescens*, P83415), HaCec (*H. armigera*, AAX51304), BmCecA (*B. mori*, NM_001043997), BmCecC1 (*B. mori*, NM_001043565), BmCecE (*B. mori*, NM_001043927), HcCecA (*H. cecropia*, P01507).

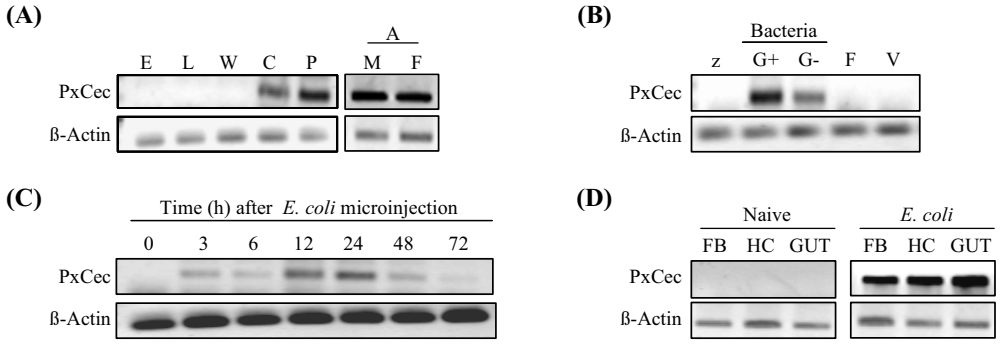


Fig. 3. Expression patterns of a cecropin gene of *P. xylostella* (PxCec) analyzed by RT-PCR. (A) PxCec expression during development at egg (E), larval (L), wandering (W), cocooning (C), pupal (P), and male (M)/female (F) adult (A) stages without any immune challenge. (B) Specific expression of PxCec against different pathogens of Gram-positive (G+, *B. subtilis*), Gram-negative (G-, *E. coli*) bacteria, fungi (F), and virus (V). Third instar larvae were injected with $5x10^4$ bacterial cells, $2x10^6$ spores of a fungus (*J. dubia*) or $5x10^5$ colony forming units of a baculovirus (*A. californica* multiple polyhedrosis virus). After 24 h post infection at 25°C, the total RNAs were extracted from the treated larvae. (C) Time course of PxCec expression after the bacterial challenge. (D) Expression of PxCec in different tissues of *P. xylostella* after the bacterial challenge. Fat body (FB), hemocytes, (HC), and gut (GUT) were collected after 24 h post infection. Expression of β-actin was tested to confirm the cDNA prepapration in all samples.

Finally, the Gly residue at the C-terminus is assumed to be amidated by peptidylglycine α-amidating enzyme to increase antibacterial activity (Hara *et al*., 1994). After these modifycations, a putative PxCec would be 39 amino acids and has Trp at the second N-terminal amino acid. An N-terminal Trp residue is present in the first or second position of most lepidopteran and dipteran cecropins but is absent in cecropin D from the silkworm, *Bombyx mori*, and in mosquito cecropins (Yang *et al*., 1999). In addition to a variation in primary amino acid sequences of cecropins among different insect orders, the modifications may contribute to expand activity spectrum of each of these molecules and to maintain structural stability (Bulet and Stocklin, 2005).

Relatively frequent positive amino acid residues ('R' and 'K') of PxCec suggest that it can bind negative charged bacterial cell membrane to exert its antibacterial activity (Hong *et al*., 2003). It is widely believed that the cationic nature of antimicrobial peptides promotes their association with anionic bacterial cell membrane and that the association with the membranes is followed by various types of membrane-invasive activities (Zasloff, 2002), including folding-insertion reactions (Silvestro and Axelsen, 2000), channel formation (Christensen *et al*., 1988), pore formation (Silvestro *et al*., 2000), and complete structural disruption (Shai, 1999). This antimicrobial activity is predicted by the structural characters of cecropins. They are composed of a strongly basic N-terminal region for binding to the bacterial membranes and a long hydrophobic C-terminal stretch to form two short α-helices that facilitate the membrane-invasive activity (Plunkett *et al*., 2009).

Expression of PxCec was inducible in response to bacterial challenge during larval stage, while it was not observed against fungal or viral infection. The bacterial challenge induced PxCec expression as early as 3 h after the injection. This is a consistent pattern like those of other cecropins A and B, which exhibit a relatively early expression pattern compared to cecropins D and E (Yamano *et al*., 1994; Hong *et al*., 2008). This inducible nature of cecropin genes is explained by the presence of conserved promoter elements including a kB-like motif, a GATA motif and R1 in *Drosophila melanogaster*

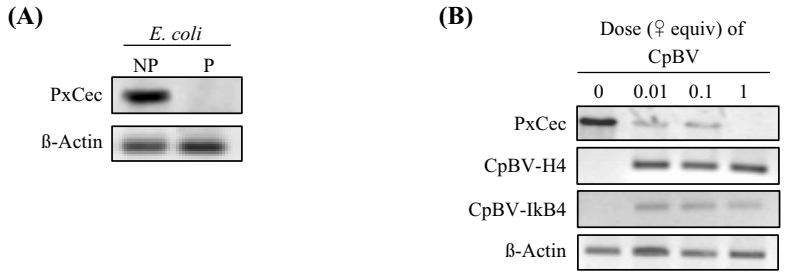


Fig. 4. Influence of parasitization by *C. plutellae* on the expression of a cecropin gene of *P. xylostella* (PxCec). All expressions were analyzed by RT-PCR. (A) Suppression of PxCec expression in the parasitized *P. xylostella* after bacterial challenge for 24 h. (B) Suppression of PxCec expression by CpBV. The viral particles were freshly collected from female ovarian calyx and the resulting extracts were quantified as female equivalent (♀ equiv) unit. Third instar larvae of *P. xylostella* were injected with different viral doses and incubated for 24 h at 25°C. Then the larvae were subjected to the bacterial challenge with *E. coli.* To confirm the viral gene expression, CpBV-H4 and CpBV-IkB4 were tested along with expression analysis of PxCec. Expression of β-Actin was tested to confirm the cDNA preparation in all samples.

(Roos *et al*., 1998; Petersen *et al*., 1999). All these promoter elements are also found in promoter of lepiodopteran cecropins (Hong *et al*., 2008). Using mutant lines of *D. melanogaster*, it has been known that the expression of the cecropin gene is induced via immune deficiency (*imd*) signal pathway (Tingvall *et al*., 2001). In *Drosophila*, *imd* pathway plays a role in mediating immune signal in response to Gram-negative bacteria or lipopolysaccharide, while Toll pathway mediates immune signal to Gram-positive bacteria or fungi (Brennan and Anderson, 2004). In this study, PxCec was expressed in response to both Gram-positive (*B. subtilis*) and negative (*E. coli*) bacteria. Recent analysis of antimicrobial peptides indicates that meso-diaminopimelic acid-containing peptidoglycans from Gram-positive and a subclass of Gram-positive bacteria including *Bacillus* species use *imd* pathway (Tanaka *et al*., 2009).

Surprisingly, PxCec expression occurs at pupal and adult developmental periods without any microbial challenge. This developmental effect on expression of cecropin is known in cecropin E of *B. mori*, where the ceceropin is expressed in egg and pupal stages without any bacterial challenge (Hong *et al*., 2008). Though we do not know the physiological significance of the developmental expression of PxCec, it is noticeable that knockdown of expression of another antimicrobial peptide, hemolin, results in abnormal larval to pupal metamorphosis of *H. cecropia* (Bettencourt *et al*., 2002). Another significance may be considered by the fact that the antibacterial peptides may protect insects from infection of potent pathogens during metamorphosis in the gut. Several antimicrobial peptides are synthesized by the midgut epithelium and released in the lumen during the larval-pupal metamorphosis of *Manduca sexta* (Russell and Dunn, 1996). The physiological significance of PxCec expression during pupal and adult stages needs to be clarified.

The expression of PxCec was suppressed by parasitization of *C. plutellae*. The suppression was also mimicked by injection of CpBV. Thus these suggest that *C. plutellae* prevents the expression of PxCec by its symbiotic virus. In addition, a previous study showed that expression of lysozyme of *P. xylostella* is inhibited by parasitization of *C. plutellae* (Bae and Kim, 2004). Why are these humoral response proteins inhibited by the parasitization or the polydnavirus infection when these enzymes can be expected to have little activity against the virus, wasp eggs or larvae, even at very high concentrations? CpBV is a polydnavirus containing about 470 kb genome, which possesses more than 130 putative open reading frames (Kim *et al*., 2007). These genes are classified into several families including Inhibitor of NF-kB (IkB)-like gene family (Kim *et al*., 2006). Eight CpBV-IkB genes have been analyzed in terms of their immunosuppressive actions against viral infection (Bae and Kim, 2009). CpBV-IkBs consist of only ankyrin repeat domains without any N- and C-terminal regulatory domains. Considering the fact that the regulatory domains undergo phosphorylation and ubiquitination for subsequent degradation to allow NF-kB to be transported into nucleus (Baeuerle, 1998), the CpBV-IkBs may act as an irreversible inhibitor to NF-kB signal pathway. In fact, *M. demolitor* bracoviral IkBs with only four ankyrin repeats can bind NF-kB proteins of *D. melanogaster* (Thoetkiattikul *et al*., 2005). This study showed the expression of CpBV-IkB4 in the larvae injected with CpBV. These facts suggest that the suppression of PxCec expression in response to bacterial challenge is explained by the inhibitory activity of CpBV-IkB inhibiting the translocation of NF-kB into nucleus. This possibility should be explored. Disruption of NF-kB signaling is likely significant to more than suppression of antimicrobial peptides because the signal pathway may be associated with encapsulation or antiviral response of insects (Zambon *et al*., 2005).

These results showed that a cecropin gene is found in *P. xylostella* and that its expression is regulated by development and immune challenge. This study also suggests that the PxCec expression is suppressed by CpBV to induce an immunosuppressive condition, which is favored by the wasp host for its successful parasitization.

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