J. Microbiol. Biotechnol. (2009), **19**(6), 610–615
doi: 10.4014/jmb.0808.495
First published online 20 December 2008

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

A Technique of Segment Expression and RNA Interference (SERI) Reveals a Specific Physiological Function of a Cysteine-Rich Protein Gene Encoded in *Cotesia plutellae* Bracovirus

Barandoc, Karen and Yonggyun Kim*

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

Received: August 29, 2008 / Revised: October 7, 2008 / Accepted: October 16, 2008

As a provirus, polydnavirus has a segmented DNA genome on chromosome(s) of host wasp. It contains several genes in each segment that presumably play critical roles in regulating physiological processes of target insect parasitized by the wasp. A cysteine-rich protein 1 (CRP1) is present in the polydnavirus *Cotesia plutellae* bracovirus (CpBV) genome, but its expression and physiological function in *Plutella xylostella* parasitized by the viral host *C. plutellae* is not known. This CpBV-CRP1 encoding 189 amino acids with a putative signal peptide (20 residues) was persistently expressed in parasitized *P. xylostella* with gradual decrease at the late parasitization period. Expression of CpBV-CRP1 was tissue-specific in the fat body/epidermis and hemocyte, but not in the gut. Its physiological function was analyzed by inducing transient expression of a CpBV segment containing CpBV-CRP1 and its promoter, which caused significant reduction in hemocyte -spreading and delayed larval development. When the treated larvae were co-injected with double-stranded RNA of CpBV-CRP1, the expression of CpBV-CRP1 disappeared, whereas other genes encoded in the CpBV segment was expressed. These co-injected larvae significantly recovered the hemocyte-spreading capacity and larval development rate. This study reports that CpBV-CRP1 is expressed in *P. xylostella* parasitized by *C. plutellae* and its physiological function is to alter the host immune and developmental processes.

Keywords: Bracovirus, *Cotesia plutellae*, CRP, hemocyte, immune, *Plutella xylostella*, polydnavirus

---

Cotesia plutellae (=*vestalis*) is a solitary braconid wasp that parasitizes the diamondback moth *Plutella xylostella*, causing an immune suppression and developmental alteration [1]. The parasitism involves release of several parasitic factors including polydnavirus (PDV), ovarian protein, and teratocyte, which exhibit a cooperative immunosuppressive effect [2]. PDV is a symbiotic DNA virus found in some endoparasitoid wasps and classified as ichnovirus and bracovirus depending on the host family and viral morphology [22]. In the parasitized host, PDVs express their own genes and then play significant roles in altering the host physiological processes [23].

The bracovirus that *C. plutellae* (CpBV) carries has a genome size of about 470 kb with at least 27 segments [13]. A full genome sequencing project on CpBV accomplished complete sequencing of 24 segments representing more than 70% of the whole CpBV genome [15]. The largest gene family of CpBV is protein tyrosine phosphatase (PTP), which consists of at least 36 putative genes [10]. The CpBV-PTPs like other PTP genes identified from various PDVs caused inhibition of cellular immune reaction [11]. The next largest CpBV gene family encodes the viral Ikb with significant expression during the entire period of parasitization, especially in midgut epithelium, in which antiviral activity appeared to be significantly depressed [14]. Other coding genes were identified, like EP1-like proteins similar to *C. congregata* bracovirus and CrV2.0 protein of *C. kariyai* bracovirus [16]. Genes encoding histone H4, viral lectin, and a gene containing the Duffy binding-like domain are encoded in the CpBV genome and contribute to the immunosuppression and developmental alteration of the parasitized host *P. xylostella* [8, 9, 18]. Two homologous genes are also identified in the CpBV genome (CpBV15α/β), in which CpBV15β inhibits hemocyte-spreading and F-actin growth in the hemocytes of parasitized host [19]. However, more than 50% predicted CpBV genes are not known in their physiological functions, owing to no or little match to any known genes even though they can exhibit significant expressions in parasitized host.

Here, we identified a CpBV gene at segment S33, named cysteine-rich protein 1 (CRP1) because of its high sequence similarity with other CRPs found in some *Cotesia* BVs.

---

*Corresponding author
Phone: +82-54-820-5638; Fax: +82-54-823-1628;
E-mail: hosanna@andong.ac.kr

However, any physiological function of these CRP genes was not reported. This study analyzed CpBV-CRP1 expression and performed a functional analysis by transient expression of CpBV-CRP1 through segment S33 injection and by subsequent specific knockdown of CpBV-CRP1 expression through RNA interference. This segment expression and RNA interference (SERI) technique revealed a physiological function of CpBV-CRP1 to be as a parasitic factor inducing a significant alteration on immune and developmental processes of the target insect. This study also proposes that this SERI technique can be applied to determine physiological functions of other hypothetical genes encoded in PDVs.

MATERIALS AND METHODS

Insect Rearing and Parasitization

P. xylostella larvae were reared under 25±1°C and a 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 condition. Then, the parasitized larvae were fed cabbage leaves and incubated at the rearing environment. After emergence, adult wasps were allowed to mate for 24 h and then used for parasitization.

CpBV Segment Isolation and Cloning

Genomic segment S33 of the CpBV genome was kindly provided by Prof. Yeon Ho Je from Seoul National University. The genomic segment was isolated based on the Tn7 transposition cloning technique using a plasmid capture system that made use of a donor vector containing an *Escherichia coli* origin of replication for amplification and an ampicillin-resistant gene for selection between Tn7 left and right ends, which would then be inserted into a target circular DNA molecule by transposition reaction using TnsABC* transposase. The reacted DNA was cloned and amplified in *E. coli.*

RNA Extraction and cDNA Construction

P. xylostella larvae parasitized by *C. plutellae* were collected after 24 h. Total RNAs were extracted from the collected larvae using Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.) followed by isopropanol precipitation. The resulting RNA pellet was washed with 70% ethanol and resuspended in DEPC-treated water. Total RNA (1 μg) was reverse-transcribed with a Maxime RT-PCR premix (Intron, Daejeon, Korea). cDNA constructed by this procedure was used for subsequent RT-PCRs. cDNAs from different tissues (gut, fat body/epidermis, and hemocyte) of the parasitized *P. xylostella* were prepared following the same procedure as above.

RT-PCR of CpBV-CRP1

Gene-specific primers were designed based on a putative open reading frame (ORF) sequence of CpBV-CRP1: forward, 5'-ATG AAC CTC TGG AAG ATG GTT GC-3' and reverse, 5'-CTT AAA ACT ATC ATC AGA GTA CGT GCA C-3'. CpBV-CRP1 amplification was performed in 35 cycles (1 min at 94°C, 45 sec at 50°C, and 2 min at 72°C). ORF 3303 and ORF 3307 were amplified under the same PCR conditions using gene-specific primers: ORF 3303 forward primer (5'-ATG AAC CTC TGG AAG ATG GTT GC-3'), ORF 3303 reverse primer (5'-GAG TAC GAC CAC GTA ATG-3'), ORF 3307 forward primer (5'-ATG GCT GAC CGC AAG CAA ACG AC-3'), and ORF 3307 reverse primer (5'-CTT CTT ACG GTT ACG GAT AGC-3').

Nucleotide and Amino Acid Sequence Analyses

Prediction of the ORF was done using the ORF Finder program of NCBI (http://www.ncbi.nlm.nih.gov). Sequence homology search with known genes of the predicted ORF of CRP was done using the BLAST program of NCBI. Signal peptide and phosphorylation site were predicted using EXPASY (http://www.expasy.ch/cgi_bin/pi_tool) and ProScan (http://npsa-pbil.ibcp.fr/tmp/2b8af505fdf6.PROSCAN) database Web engines, respectively. Alignment of CpBV-CRP1 with other known CRPs from *Cotesia* family members and some species was performed using the DNAstar program (Version 5.01; DNAstar Inc., Madison, U.S.A.).

Segment Expression and RNA Interference (SERI)

For transient expression of CpBV segment S33, genomic DNA of this segment was mixed with Metafectene PRO transfection reagent (Biontex, Plannegg, Germany). Briefly, 0.5 μg of CpBV-S33 was mixed with 3 μl of transfection reagent and incubated for 20 min at room temperature to allow DNA-lipid complexes to be formed before injection into the hemocoel of second instar *P. xylostella.* Glass capillary (World Precision Instruments, Sarasota, FL, U.S.A.) injection needles were made using a Micropipette puller PN-30 (Narishige, Japan). The DNA-transfection reagent complex (60 nl) was injected to each larva at a rate of 10 nl/sec using an UltraMicroPump (four) with SYS-microcontroller (World Precision Instruments, Sarasota, FL, U.S.A.). Microinjection was performed under a microscope (Olympus S730, Japan). The same technique was used to inject double-stranded RNA (dsRNA) of CpBV-CRP1 for RNA interference (RNAi). The success of transfection was analyzed by RT-PCR using gene-specific primers.

RNAi was performed using dsRNA, which was prepared using a Megascript RNAi kit (Ambion, TX, U.S.A.) according to the manufacturer's instruction. A dsRNA with a size of 200 bp was prepared using two gene-specific primers: forward, 5'- GAC GAA TAC AGC AAG CTG ATC G-3' and reverse, 5'-CTT AAA ACT ATC AGA GTA CGT GCA C-3'. The PCR product (200 bp) was cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA, U.S.A.). Two separate clones with the same target region (i.e., CpBV-CRP1) but different orientations were confirmed and analyzed before linearization by restriction enzyme digestion using BamHI. After digestion, the two linearized DNA templates were used for *in vitro* transcription reactions. Sense and antisense strands were synthesized using T7 RNA polymerase. Annealing of both strands to form dsRNA was performed by heating the reaction to 75°C for 5 min followed by mixing and cooling to room temperature. DNA templates and single-stranded RNA were digested using DNaseI and RNase, respectively. The resulting dsRNA was purified and eluted in elution buffer (10 mM Tris/HCl, pH 7.0, 1 mM EDTA).

Hemolymph Collection and Hemocyte-Spreading Assay

Hemolymph from larvae injected with CpBV segment S33, dsRNA of CpBV-CRP1, or non-injected *P. xylostella* was collected for hemocyte-spreading assay [19]. Briefly, 15 larvae were surface-sterilized and the hemolymph was collected in 150 μl of an anticoagulant buffer that was prepared fresh by dissolving 4 mg of L-cysteine hydrochloride (Sigma, MO, U.S.A.) in 5 ml of 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]. Fifty μl of this suspension was used to prepare hemolymph monolayer and left in a moist chamber at 23°C for 45 min. The spread hemocytes

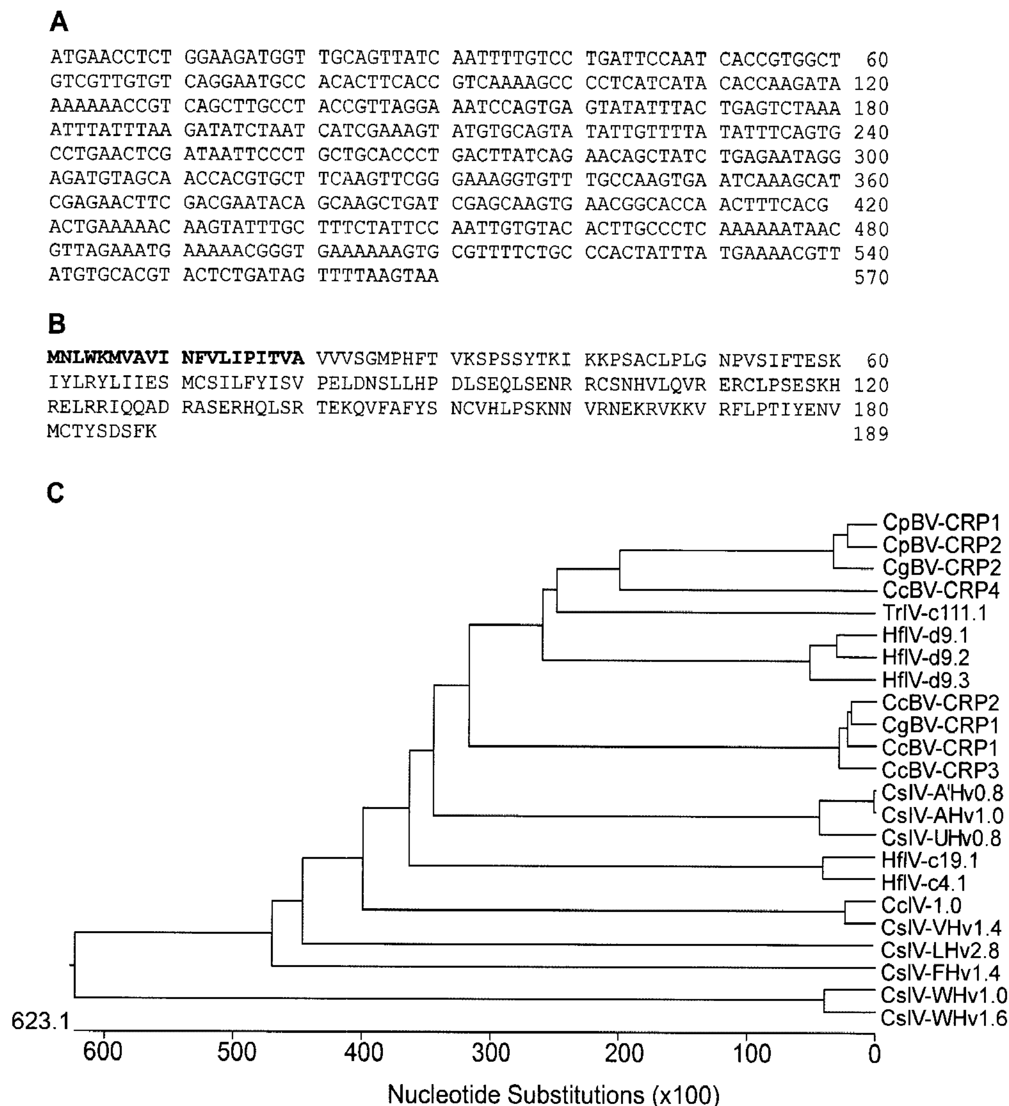

Fig. 1. CpBV-CRP1 and other cysteine-rich protein genes encoded in polydnaviruses.
(**A**) Nucleotide and (**B**) deduced amino acid sequence of CpBV-CRP1. Signal peptide is predicted and denoted in bold letters. **C.** A phylogenetic tree that shows amino acid sequence alignment of CpBV-CRP1 with other CRP genes from bracovirus (BV) and ichnovirus (IV). All sequences were obtained with NCBI accession numbers in parentheses: CcBV-CRP1 (YP184890), CcBV-CRP2 (CAG17462), CcBV-CRP3 (YP184890), CcBV-CRP4 (YP184906), CgBV-CRP1 (AAR37024), CgBV-CRP2 (AAR37022), CpBV-CRP1 (AAV98033), CpBV-CRP2 (AAY22183), CcIV1.0 (BAC55881), CsIV-AHv1.0 (AAO43442), CsIV-A'Hv0.8 (AAO43443), CsIV-FHv1.4 (AAO43444), CsIV-LHv2.8 (AAO43445), CsIV-UHv0.8 (AAO43447), CsIV-VHv1.4 (YP589077), CsIV-WHv1.0 (YP589078), CsIV-WHv1.6 (YP589079), HfIV-c4.1 (YP001031276), HfIV-c19.1 (YP001031361), HfIV-d9.1 (YP001031331), HfIV-9.2 (YP001031333), HfIV-d9.3 (YP001031334), and TrIV-c111.1 (BAF45771).

were characterized by the presence of filopodial extension. The percentage of spread hemocytes was scored by randomly counting 100 hemocytes from 10 selected microscopic fields at 400× magnification under a phase-contrast microscope (BX41; Olympus, Japan). Each treatment was independently replicated three times.

Bioassay of Larval Development

Early third instar larvae (5 days old after oviposition at 25°C) were micro-injected with CpBV segment S33 and/or dsRNA of CpBV-CRP1. Thirty live larvae were chosen from each treatment and maintained at the rearing conditions. Each treatment was independently replicated three times. These treated larvae were assayed at 5 days after injection to determine cocoon formation for subsequent pupation. Non-injected early third instar larvae served as control.

Statistical Analysis

Treatment means and variances were analyzed using one-way ANOVA by PROC GLM of the SAS program [20]. All means were compared by the least-squared difference (LSD) test at Type I error=0.05.

Results

Prediction of CpBV-CRP1

A putative gene was predicted to be CpBV-CRP1 (ORF 3304 in Fig. 3A) from a genome sequencing project of CpBV (Fig. 1A). It encodes a sequence of 189 amino acids with a putative signal peptide (Fig. 1B). It shares 59.8%,

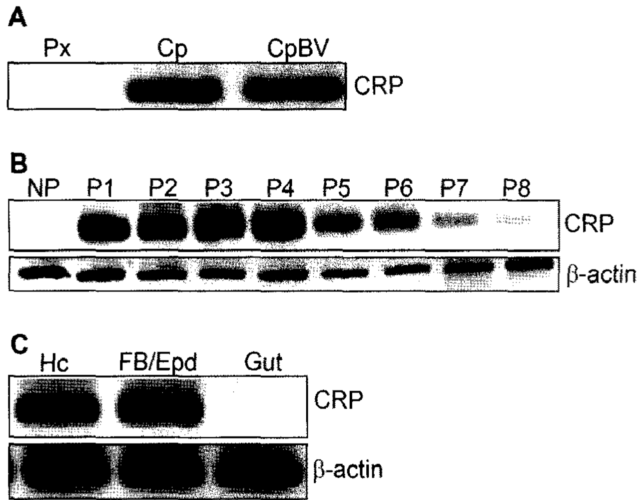

Fig. 2. Expression of CpBV-CRP1 in *Plutella xylostella* (Px) parasitized by *Cotesia plutellae* (Cp).
A. Demonstration of CpBV-CRP1 encoded in the CpBV genome by PCR using genomic DNAs of *P. xylostella*, *C. plutellae*, and CpBV as templates. **B.** Expression of CpBV-CRP1 in different ages of the parasitized *P. xylostella*. **C.** Tissue specificity of CpBV-CRP1 expression in hemocyte (Hc), gut, and fat body/epidermis (FB/Epd) of the parasitized *P. xylostella*. RT-PCR using primers for β-actin served as control.

28.1%, and 69.6% sequence homologies with CpBV-CRP2 (ORF 3303 in Fig. 3A), *C. congregata* CRP1, and *C. glomerata* CRP2, respectively (Fig. 1C). The phylogenetic tree showed that these bracoviral CRP genes showed clear relationship with ichnoviral cys-motif genes. However, Blast search of CpBV-CRP1 from the GenBank of NCBI did not show any clear match with genes known in biological function, although it had a slight homology (E value=0.81) with RNA helicase of *Salmonella enterica* (Accession No: ZP 02706090).

Expression of CpBV-CRP1 in Parasitized *P. xylostella*

The predicted CpBV-CRP1 is encoded in *C. plutellae* and CpBV genomes (Fig. 2A) owing to its proviral nature. Its expression was consistently observed in all ages of the parasitized *P. xylostella* (Fig. 2B). However, its expression appeared to decrease with wasp parasitoid development. Its expression was tissue-specific (Fig. 2C). Hemocytes and fat body showed its expression, but gut did not.

Parasitic Functions of CpBV-CRP1 Expression

To understand its expression on the physiological alteration of *P. xylostella*, we used an individual segment expression technique. CpBV-CRP1 is encoded in segment S33 of CpBV (Fig. 3A). When this segment was micro-injected into the second instar larvae of nonparasitized *P. xylostella*, CpBV-CRP1 expression was detected from 12 h to 72 h after the treatment (Fig. 3B). Moreover, co-injection of dsRNA that is specific to CpBV-CRP1 with segment S33 could knockdown the expression of CpBV-CRP1 (Fig. 3C). This dsRNA did

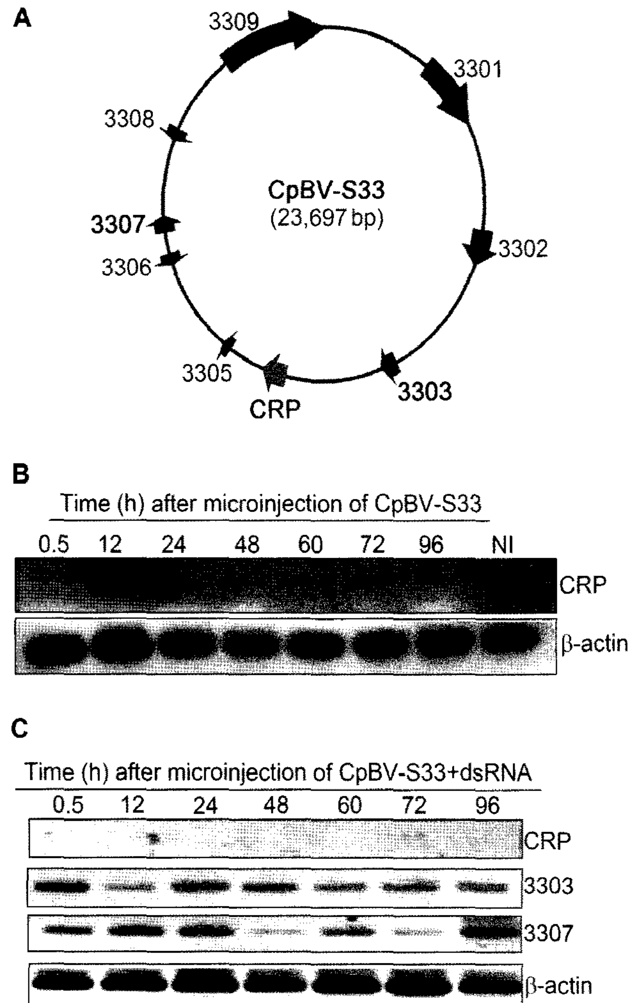

Fig 3. Segment expression of CpBV-S33 in nonparasitized second instar larvae of *Plutella xylostella* and a specific knockdown of CpBV-CRP1 (CRP) by its double-stranded RNA (dsRNA).
A. A segment map of CpBV-S33 showing nine predicted open reading frames in arrow directions. **B.** RT-PCR showing expression of CpBV-CRP1 at different time points post-injection of CpBV-S33, compared with noninjected larvae (NI). **C.** Co-injection of CpBV-S33 and dsRNA and RT-PCR showing knockdown of CpBV-CRP1 expression with significant expressions of nontargets (3303 and 3307 genes). β-Actin was used as the positive control of RT-PCR.

not influence the expression of other encoded genes like ORF 3303 and ORF 3307.

With this individual segment expression technique, we tested the effects of CpBV-CRP1 on the cellular immune capacity and larval development of *P. xylostella* (Fig. 4). Transient expression due to segment S33 injection significantly inhibited hemocyte-spreading behavior of the treated larvae at 12 h post injection (Fig. 4A). To test this inhibition due to CpBV-CRP1, dsRNA was co-injected and the hemocyte spreading behavior recovered up to ≈83% compared with untreated control. Similar analysis was applied to larval development measured by cocoon formation rate during 5 days after the treatment (Fig. 4B). All treated larvae formed cocoons

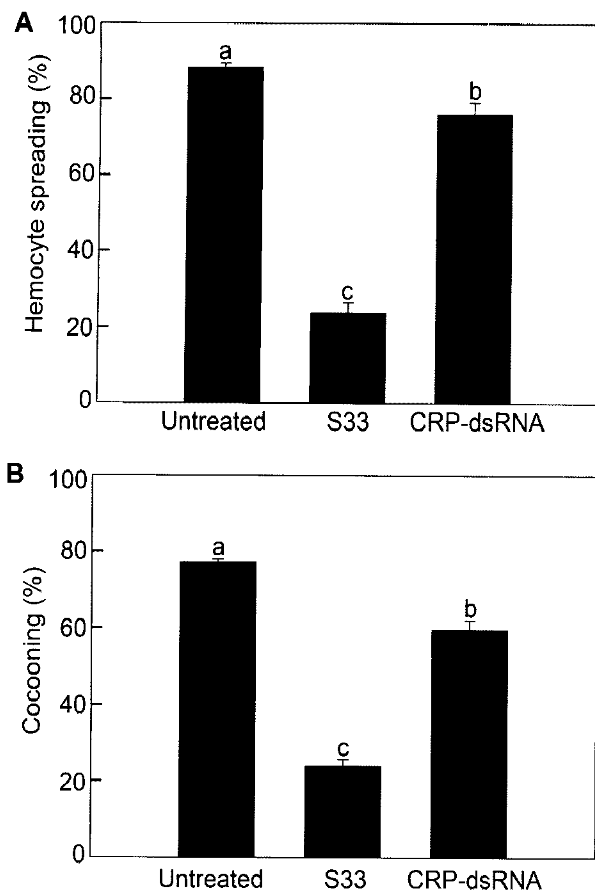

Fig. 4. Effects of CpBV-CRP1 expression on hemocyte-spreading behavior and larval development of *Plutella xylostella*. **A.** Inhibition of hemocyte-spreading analyzed at 24 h after microinjection of CpBV-S33 (S33) or its mixture with double-stranded RNA (CRP-dsRNA). **B.** Delay of larval development by segment S33 expression and its recovery by knockdown of CpBV-CRP1 expression using CRP-dsRNA. Larval development was analyzed by measuring cocooning rate at 5 days after the micro-injection treatment. Each treatment was independently replicated three times. Different letters above standard deviations indicate significant differences among means at Type I error=0.05 (LSD test).

but showed different developmental rates. S33-injected larvae showed a delayed larval development and resulted in only 23.9% cocoon formation rate during the predetermined period. However, dsRNA of CpBV-CRP1 significantly rescued the altered development up to ≈80% compared with untreated control.

Discussion

Several PDV genome studies support independent origins of IV and BV, owing to lack of shared common viral genes [24]. Exceptions are the IkB and CRP gene families [5]. In this study, a phylogenetic analysis of all bracoviral CRPs and ichnoviral cys-motif genes also proved clear relationship between these two viral groups. Some cys-motif genes have been known to play significant parasitic roles by inducing both host immunosuppression and developmental alteration mainly by inhibiting host protein translation [7, 12, 17]. However, no physiological functions were known in CRPs derived from BVs.

The persistent and tissue-specific expression patterns of CpBV-CRP1 suggest critical role(s) in the parasitism of *C. plutellae* to *P. xylostella*. Even though there is little sequence similarity between CpBV-CRP1 and ichnoviral cys-motif genes, this study demonstrated its parasitic roles in altering host immune and developmental processes, by a SERI technique. To apply the SERI technique, segment S33 encoding CpBV-CRP1 was specifically cloned in *E. coli* based on Tn7 transposition by a plasmid capture system [3]. Micro-injection of CpBV-S33 into nonparasitized *P. xylostella* showed clear expression of CpBV-CRP1 as well as other encoded genes like ORF 3303 and ORF 3307. Similar transient expression was used to address the physiological functions of CpBV-PTP [10], CpBV-ELP1 [16], and CpBV-H4 [8] using a eukaryotic expression vector by cloning each gene under polyhedrin or immediate early expressed promoters. Thus, the difference of this PDV segment expression compared with earlier transient expression studies is to express CpBV-CRP1 in *P. xylostella* using its own viral promoter. However, the segment injection allows expressions of other encoded genes in the segment. To overcome this compounding effect and show the specific effect of CpBV-CRP1, its specific RNA interference was accomplished by co-injecting its specific dsRNA, at which expressions of other encoded genes were maintained. Specifically, expression of ORF 3303 was not inhibited by dsRNA of CpBV-CRP1. ORF 3303 is another CRP in CpBV due to its sequence homology with CpBV-CRP1. Maintaining its expression level under dsRNA of CpBV-CRP1 indicates the specificity of the RNA interference. Under this specific RNA interference, the treated larvae exhibited significant recovery of both physiological processes suppressed by segment expression. However, this study does not explain how CpBV-CRP1 inhibits hemocyte-spreading and delays host larval development. Considering the significant effects of VHv1.4 on inhibition of actin polymerization [4] or on specific translational control of host mRNAs [12] to induce immunosuppression and developmental alteration, another SERI experiment can be applied to address whether CpBV-CRP1 may interrupt F-actin growth or impair translational efficacy of mRNAs associated with host development, like juvenile hormone esterase.

It is noteworthy that the dsRNA treatment resulted in ≈80% in recovery efficiency, which was significantly different compared with physiological competency of untreated larvae. Here, we interpret that this difference was probably due to the contribution of other encoded genes. Their influence on the physiological alterations by CpBV-CRP1 may be assessed by their specific SERI or by a mixture treatment of their specific dsRNAs after micro-injection of CpBV-S33.

There are increasing numbers of many hypothetical PDV genes predicted from several genome studies [6, 15, 21, 24]. However, the physiological end-points disrupted by these PDV genes appear to be overlapped and mainly concentrate on immune and developmental processes [23]. It may be needed to understand a target physiological end-point interrupted by a hypothetical PDV gene, which would be followed by identification of its ultimate molecular target(s) involved in the physiological process. Under these considerations, the SERI technique would be a fast and convenient screening method in the PDV research area. This study addressed two physiological functions of CpBV-CRP1 in *C. plutellae* parasitism by the SERI technique.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-313-F00009). Second stage BK21 supported K. Barandoc during this work.

REFERENCES

1. Bae, S. and Y. Kim. 2004. Host physiological changes due to parasitism of a braconid wasp, *Cotesia plutellae*, on diamondback moth, *Plutella xylostella*. *Comp. Biochem. Physiol.* **138A:** 39–44.
2. Basio, N. A. and Y. Kim. 2006. Additive effect of teratocytes and calyx fluid from *Cotesia plutellae* on immunosuppression of *Plutella xylostella*. *Physiol. Entomol.* **31:** 341–347.
3. Choi, J. Y., J. Y. Roh, J. W. Kang, H. J. Shim, S. D. Woo, B. R. Jin, M. S. Li, and Y. H. Je. 2005. Genomic segments cloning and analysis of *Cotesia plutellae* polydnavirus using plasmid capture system. *Biochem. Biophys. Res. Commun.* **332:** 487–493.
4. Cui, L., A. Soldevila, and B. A. Webb. 1997. Expression and hemocyte-targeting of a *Campoletis sonorensis* polydnavirus cysteine-rich gene in *Heliothis virescens* larvae. *Arch. Insect Biochem. Physiol.* **36:** 251–271.
5. Dupuy, C., E. Huguet, and J. D. Drezen. 2006. Unfolding the evolutionary story of polydnaviruses. *Virus Res.* **117:** 81–89.
6. Espagne, E., D. Catherine, E. Huguet, L. Cattolico, B. Provost, N. Martins, M. Poirie, G. Periquet, and J. M. Drezen. 2004. Genome sequence of a polydnavirus: Insights into symbiotic virus evolution. *Science* **306:** 286–289.
7. Fath-Goodin, A., T. A. Gill, and B. A. Webb. 2006. Effect of *Campoletis sonorensis* ichnovirus cys-motif proteins on *Heliothis virescens* larval development. *J. Insect Physiol.* **52:** 576–585.
8. Gad, W. and Y. Kim. 2002. A viral histone H4 encoded by *Cotesia plutellae* bracovirus inhibits haemocyte-spreading behaviour of the diamondback moth, *Plutella xylostella*. *J. Gen. Virol.* **89:** 931–938.
9. Ibrahim, A. M. A., J. Y Choi, Y. H Je, and Y. Kim. 2005. Structure and expression profile of two putative *Cotesia plutellae* bracovirus genes (CpBV-H4 and CpBV-E94α) in parasitized *P. xylostella*. *J. Asia-Pac. Entomol.* **8:** 359–366.
10. Ibrahim, I. M. A. and Y. Kim. 2007. Protein tyrosine phosphatases of *Cotesia plutellae* bracovirus inhibit insect cellular immune responses. *Naturwissenchaften* **95:** 25–32.
11. Ibrahim, A. M. A. and Y. Kim. 2008. Protein tyrosine phosphatases of *Cotesia plutellae* bracovirus inhibit insect cellular immune responses. *Naturwissenschaften* **95:** 25–32.
12. Kim, Y. 2005. Identification of host translation inhibitory factor of *Campoletis sonorensis* ichnovirus on the tobacco budworm, *Heliothis virescens*. *Arch. Insect Biochem. Physiol.* **59:** 230–244.
13. Kim, Y. 2006. Polydnavirus and its novel application to insect pest control. *Kor. J. Appl. Entomol.* **45:** 241–259.
14. Kim, Y., N. A. Basio, A. M. A. Ibrahim, and S. Bae. 2006. Gene structure of *Cotesia plutellae* bracovirus (CpBV)-IkB and its expression pattern in the parasitized diamondback moth, *Plutella xylostella*. *Kor. J. Appl. Entomol.* **45:** 15–24.
15. Kim, Y., J. Y. Choi, and Y. H. Je. 2007. *Cotesia plutellae* bracovirus genome and its function in altering insect physiology. *J. Asia-Pac. Entomol.* **10:** 181–191.
16. Kwon, B. and Y. Kim. 2008. Transient expression of an EP1-like gene encoded in *Cotesia plutellae* bracovirus suppresses the hemocyte population in the diamondback moth, *Plutella xylostella*. *Dev. Comp. Immunol.* **32:** 932–942.
17. Li, X. and B. A Webb. 1994. Apparent functional role for a cysteine-rich polydnavirus protein in suppression of the insect cellular immune response. *J. Virol.* **68:** 7482–7489.
18. Nalini, M., J. Y. Choi, Y. H. Je, I. Hwang, and Y. Kim. 2008. Immunoevasive property of a polydnaviral product, CpBV-lectin, protects the parasitoid egg from hemocytic encapsulation of *Plutella xylostella* (Lepidoptera: Yponomeutidae). *J. Insect Physiol.* (In press.)
19. Nalini, M. and Y. Kim. 2007. A putative protein inhibitory factor encoded in *Cotesia plutellae* bracovirus suppresses host hemocyte-spreading behavior. *J. Insect Physiol.* **53:** 1283–1292.
20. SAS Institute, Inc. 1989. *SAS/STAT User's Guide*, release 6.03 ed. SAS Institute, Cary, NC.
21. Tanaka, K., R. Lapointe, W. E. Barney, A. M. Makkay, D. Stoltz, M. Cusson, and B. A. Webb. 2007. Shared and species-specific features among ichnovirus genomes. *Virology* **363:** 26–35.
22. Webb, B. A. 1998. *Polydnavirus Biology, Genome Structure, and Evolution*, pp. 105–139. *In* L. K. Miller and L. A. Ball (eds.), *The Insect Viruses*. Plenum, New York.
23. Webb, B. A. and M. R. Strand. 2005. *The Biology and Genomics of Polydnaviruses*, pp. 260–323. *In* L. I. Gilbert, K. Iatrou, and S. S. Gill (eds.), *Comprehensive Molecular Insect Science*, Vol. 5. Elsevier Press, San Diego.
24. Webb, B. A., M. R. Strand, S. E. Dickey, M. H. Beck, R. S. Hilgarth, W. E. Barney, *et al.* 2006. Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* **347:** 160–174.