Characterization of joining sites of a viral histone H4 on host insect chromosomes

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Abstract

A viral histone H4 (CpBV-H4) is encoded in a polydnavirus, Cotesia plutellae bracovirus (CpBV). It plays a crucial role in parasitism of an endoparasitoid wasp, C. plutellae, against diamondback moth, Plutella xylostella, by altering host gene expression in an epigenetic mode by its N-terminal tail after joining host nucleosomes. Comparative transcriptomic analysis between parasitized and nonparasitized P. xylostella by RNA-Seq indicated that 1,858 genes were altered at more than two folds in expression levels at late parasitic stage, including 877 up-regulated genes and 981 down-regulated genes. Among parasitic factors altering host gene expression, CpBV-H4 alone explained 16.3% of these expressional changes.

To characterize the joining sites of CpBV-H4 on host chromosomes, ChIP-Seq (chromatin immunoprecipitation followed by deep sequencing) was applied to chromatins extracted from parasitized larvae. It identified specific 538 ChIP targets. Joining sites were rich (60.2%) in AT sequence. Almost 40% of ChIP targets included short nucleotide repeat sequences presumably recognizable by transcriptional factors and chromatin remodeling factors. To further validate these CpBV-H4 targets, CpBV-H4 was transiently expressed in nonparasitized host at late larval stage and subjected to ChIP-Seq. Two kinds of ChIP-Seqs shared 51 core joining sites. Common targets were close (within 1 kb) to genes regulated at expression levels by CpBV-H4. However, other host genes not close to CpBV-H4 joining sites were also regulated by CpBV-H4. These results indicate that CpBV-H4 joins specific chromatin regions of P. xylostella and controls about one sixth of the total host genes that were regulated by C. plutellae parasitism in an epigenetic mode.

Introduction

Polydnaviruses (PDVs) are a group of insect viruses symbiotic to some endoparasitoid wasps belonging to families Braconidae and Ichneumonidae. Depending on host wasp families, PDVs are divided into two genera: Ichnovirus (IV) and Bracovirus (BV) [1]. PDV genome consists of genome encapsidated into viral particles during replication and nonencapsidated genome [2]. Its nonencapsidated genome appears to be essential to the replication of encapsidated genome. Some gene products of the nonencapsidated genome are likely viral coat...
proteins [3]. The encapsidated genome in the viral coat is expressed in parasitized host. It plays crucial roles in altering host physiological processes for successful parasitism [4].

An endoparasitoid wasp, *Cotesia plutellae*, specifically parasitizes young larvae of the diamondback moth, *Plutella xylostella*. It has several parasitic factors, including a symbiotic PDV and *C. plutellae* bracovirus (CpBV) [5]. Parasitized *P. xylostella* larvae exhibit significant physiological alterations, including immunosuppression and delayed larval development [6,7]. CpBV plays a crucial role in parasitism of *C. plutellae*. Its genome contains 157 putative open reading frames (ORFs), most of which are expressed in parasitized larvae [8].

CpBV is a bracoviral PDV. Its encapsidated genome shares common genes with other BVs [9]. However, some genes encoded in the genome of CpBV are unique in *Cotesia*-associated BVs [10]. A viral histone H4 is encoded only in *Cotesia*-associated BVs and CpBV-H4 is a viral histone H4 ortholog of CpBV [11]. CpBV-H4 is highly homologous to host histone H4 except an extended N terminal tail which contains 38 amino acids with nine lysine residues [12].

CpBV-H4 suppresses host immunity by inhibiting gene expression of phenoloxidase and antimicrobial peptides [13]. CpBV-H4 expression also inhibits gene expression of insulin-like peptide of host larvae, leading to developmental retardation and hemolymph hypertrehalosemic conditions which are beneficial for symbiotic parasitoid development [14]. However, these physiological alterations by CpBV-H4 are completely lost when it loses its N-terminal extended tail [15]. These findings suggest that CpBV-H4 can modulate host gene expression in an epigenetic mode.

CpBV-H4 joins eukaryotic nucleosomes by interacting with other histone monomers to form an octamer [16]. Using a nonhost, *Tribolium castaneum*, CpBV-H4 has been transiently expressed and subsequently subjected to chromatin immunoprecipitation (ChIP), in which ChIP targets are located on the upstream or downstream of coding DNA sequences (CDSs) annotated as inducible genes [17]. Furthermore, subtractive suppressive hybridization (SSH) analysis performed in natural host, *P. xylostella*, has shown that CpBV-H4 suppresses the expression of at least 115 genes, including chromatin remodeling factors that can subsequently alter other gene expression [14]. These previous results suggest that CpBV-H4 may have structural or DNA sequence specificity to joining sites. However, the molecular characters of the incorporation sites of CpBV-H4 on natural host genome of *P. xylostella* remain unknown. In addition to SSH analysis, CpBV-H4 might up-regulate or down-regulate host gene expression. Thus, in addition to the previous ChIP assay on non-natural host and SSH on natural host, genome-wide transcriptomic analyses are needed to understand host-parasite molecular interaction that control host gene expression by CpBV-H4. Furthermore, we do not know how much host gene regulation induced by *C. plutellae* parasitism could be explained by CpBV-H4.

Therefore, the objective of this study was to determine the incorporation sites of CpBV-H4 on host chromosomes of *P. xylostella* parasitized by *C. plutellae* by ChIP-Seq analysis. CpBV-H4 was transiently expressed in nonparasitized host followed by ChIP-Seq to confirm the incorporation sites determined in parasitized host. In addition, host genes changed in expression level by CpBV-H4 were determined by comparing the transcriptome of nonparasitized host transiently expressing CpBV-H4 to that of parasitized host using RNA-Seq.

### Results

**Influence of CpBV-H4 on host gene regulation during parasitism**

*C. plutellae* alters host gene expression via parasitic factors including CpBV-H4 which has been regarded as a regulator of host gene expression at transcriptional level by an epigenetic mode [14]. To determine genes regulated only by CpBV-H4, four RNA-Seq data (S1 Table) were compared in fragment per kilobase of transcript per million mapped reads (FPKM)
levels: nonparasitized larvae (‘NP5’), parasitized larvae (‘P7’), nonparasitized larvae transiently expressing \textit{CpBV-H4} (‘vH4’), and truncated \textit{CpBV-H4} (‘vH4T’). All four transcriptomes had reads of more than 60 Gb in size. More than 50% of these sequences were mapped to \textit{P. xylostella} genome sequence with P7 having lower mapping rate (S1 Fig). Relatively low mapping ratio of P7 might be due to contaminants of immature \textit{C. plutellae} wasp and teratocyte transcripts because all reads were mapped only to \textit{P. xylostella} genome. Using these four transcriptomes, differentially expressed gene (DEG) analyses were performed between NP5 and P7 to obtain specific host genes regulated by parasitism or between vH4 and vH4T to search for specific host genes regulated only by \textit{CpBV-H4} expression (Fig 1). Expression pattern analysis showed that the number of major clades in the comparison between P7 and NP5 was higher than that between vH4 and vH4T (Fig 1A). DEG analysis between P7 and NP5 showed that 1,858 host genes exhibited more than 2-fold of changes in expression level after parasitism, including 877 up-regulated genes and 981 down-regulated genes (Fig 1B). It also showed that 1,190 host genes exhibited more than 2-fold of changes in expression level after \textit{CpBV-H4} expression by comparing the transcript levels between vH4 and vH4T treatments, including 431 up-regulated host genes and 759 down-regulated host genes.

To determine the influence of \textit{CpBV-H4} on the regulation of host genes during \textit{C. plutellae} parasitism, parasitism-specific DEG (‘P7/NP5’, 1,858 genes) and \textit{CpBV-H4} DEG (‘vH4/vH4T’, 1,190 genes) were compared (Fig 2). This comparison resulted in 302 genes overlapped between the two DEGs, including 81 up-regulated genes (Fig 2A and Table 1) and 221 down-regulated genes (Fig 2B and Table 2). Almost half of these genes up-regulated by \textit{CpBV-H4} had no functional annotation whereas almost 70% genes down-regulated by \textit{CpBV-H4} were predicted to have functions in development and metabolism. These DEG analyses indicated that \textit{CpBV-H4} expression contributed 16.3% (302/1,858 x 100) to \textit{C. plutellae} parasitism with respect to host gene regulation. In addition, \textit{CpBV-H4} appeared to control specific target genes because host genes regulated by \textit{CpBV-H4} were significantly (X$^2 = 1,804.12$; df = 10; $P < 0.0001$) different in functional categories compared to those regulated by \textit{C. plutellae} parasitism (Fig 2C).

Sequence specificity of \textit{CpBV-H4}-joining sites on host chromosomes

To determine the specificity of \textit{CpBV-H4}-joining sites on host chromosomes, ChIP-Seq analysis was performed using a polyclonal antibody raised against \textit{CpBV-H4}. ChIP isolated 1,498 targets against chromatins originated from larvae at late parasitism (‘P7’). After deleting nonspecific sites detected from ChIP-Seq analysis using chromatins originated from NP5 larvae, P7-specific ChIP targets had 538 sites (Fig 3A). Similar ChIP-Seq was performed against chromatins extracted from larvae transiently expressing \textit{CpBV-H4} and 394 targets were obtained after deleting nonspecific sites detected from ChIP-Seq analysis using larvae expressing truncated \textit{CpBV-H4}. There were 51 common core target genes (‘vH4-ChIP1 ~ vH4-ChIP51’) between P7-specific ChIP targets and \textit{CpBV-H4}-specific ChIP targets (Table 3). Genes close (within 1 kb) to these 51 core target sites were predicted to have functions in development, metabolism, immunity, signaling, and gene expression (Fig 3B).

Sequences of P7-specific ChIP targets (538 sites) were further analyzed in order to find any consensus sequences (Fig 4). For this analysis, 480 target sequences were used while the other 58 target sequences did not have unique match with \textit{P. xylostella} genome. These 480 targets were rich (60.2%) in AT content. Almost 40% targets contained two nucleotide repeat sequences such as GT- (14.9%), AC- (12.4%), CT- (6.3%), and AG- (4.6%) repeats (Fig 4A and S2 Fig). Three nucleotide repeat sequences (CAT and TGA), four nucleotide repeat sequences (TACA, TCAC, TGAG, TCTG, GTCT, and TAGA), and five nucleotide repeat sequences (TTCTG,
Fig 1. Alteration of host gene expression by parasitism of *C. plutellae* or expression of a viral gene *CpBV-H4*. To determine parasitic control of *P. xylostella* gene expression, transcripts were subjected to RNA-Seq from 4th instar larvae parasitized (‘P7’) or nonparasitized (‘NP5’) by *C. plutellae*. To determine *CpBV-H4* control of host gene expression, transcripts were subjected to RNA-Seq from 4th instar larvae transiently expressing *CpBV-H4* containing N-terminal tail (‘vH4’) or truncated *CpBV-H4* (‘vH4T’) after deleting N-terminal tail. (A) Heat map analysis of expression patterns in four different treatments. (B) DEG analysis of
CAATA, and ATTCT) were also detected (S2 Fig). GT and AC repeat-joining sites were close to genes that were up- or down-regulated in their expression during C. plutellae parasitism (Fig 4B). In contrast, CT- and AG-repeat joining sites were associated with up-regulated genes. These repeats were located in 45.8% sites at the upstream ('UP'), 20.8% sites at the downstream ('DOWN'), and 33.3% sites at the gene body ('GB') (Table 4). Among these repeats, more than 50% CT- and AG-repeats were located at GB. Most repeat motifs were likely to be associated with DNA elements recognized by transcriptional factors or chromatin remodeling factors (S2 Table). Among the 51 core targets of CpBV-H4, more than 60% possessed GT- or AC-repeats.

Physical mapping of 51 core joining sites of CpBV-H4 on host chromosomes

The 51 core joining sites of CpBV-H4 were mapped onto the chromosomes of P. xylostella (Fig 5). P. xylostella chromosomes have been characterized with 30 linkage groups and uncharacterized scaffolds [18]. Fifteen CpBV-H4 joining sites were located on 11 chromosomes while the remaining 36 CpBV-H4 sites were found on uncharacterized chromosome(s). On this mapping, 302 host DEGs regulated by CpBV-H4 during C. plutellae parasitism were applied (see vertical lines in Fig 5). These target genes were not only distributed on chromosomes containing CpBV-H4 targets, but also distributed on other chromosomes without CpBV-H4 targets.

Influence of relative locality of CpBV-H4 targets on expression of nearby genes

We then tested whether there was any positional effect of CpBV-H4 targets on regulating gene expression of nearby genes. To address this question, 15 CpBV-H4 targets (vH4-ChIP1 ~ vH4-ChIP15) were further divided into three relative localities in each nearby gene on coding DNA sequence or gene body ('GB'), upstream intergenic region ('UP'), and downstream intergenic region ('DOWN'). Subsequently, when relative localities and expressional changes of nearby genes induced by CpBV-H4 expression were compared, individual ChIPs were not evenly distributed on all tested genes. They were concentrated around target gene sites (red color in (Fig 6) except vH4-ChIP2 and vH4-ChIP3. This might be due to the fact that target genes were determined by ChIP frequency in a specific spot, not by total ChIP frequency in each locality. Host genes around CpBV-H4 targets exhibited expressional changes (either up- or down-regulated). Relative distribution of CpBV-H4 target sites was further analyzed to determine any positional effect on regulating the expression level of nearby genes (Fig 7). In this analysis, 75 neighboring genes including 15 CpBV-H4-joining sites were assessed. In these CpBV-H4 joining sites, ChIP targets were relatively evenly distributed on three positions (Fig 7A). The less number of major ChIPs by about two folds of change at GB might be due to difference in domain size because the average GB domain length (≈ 1.5 kb) was shorter by almost two folds than UP (≈ 2.5 kb) and DOWN (≈ 3.0 kb) domain lengths. Each gene was classified by the presence of the main ChIP peak among the three relative localities (S3 Table and Fig 7B). Although different genes had different locations of main ChIP peaks, the three ChIP positions did not have significant effect on the direction of gene regulation (X^2 = 0.3995; df = 2; P = 0.8189).
Fig 2. Influence of CpBV-H4 on altering host gene expression of *P. xylostella* during *C. plutellae* parasitism. (A) Genes up-regulated by both parasitism and CpBV-H4 (left panel). Gene ontology (GO) analysis of 81 commonly up-regulated genes (right panel). (B) Genes down-regulated by both parasitism and CpBV-H4 (left panel). GO analysis of 221 commonly down-regulated genes (right panel). ‘vH4/vH4T’ represents specific genes regulated by CpBV-H4 (vH4) by deducting backgroung genes induced by the truncated CpBV-H4 (vH4T). ‘P7/NP5’ represents specific genes regulated by parasitism by deducting background genes expressing under...
Discussion

Parasitism by endoparasitoid wasps using PDV alters host gene expression to allow internal environment to be favorable for parasite development [19]. Two different endoparasitoid wasps, Diadegma semiclausum and C. plutellae possessing IV and BV, respectively, can parasitize P. xylostella and significantly alter host gene expression [20, 21]. Several viral factors have been proposed to be able to regulate host gene expression in CpBV [10]. CpBV-IkB is a viral IkB possessing ankyrin repeat. However, it lacks regulatory domain and suppresses antimicrobial peptide gene expression in response to immune challenge [22]. Two homologous proteins, CpBV15α and CpBV15β, can interrupt eukaryotic translation initiation factors and act as translational inhibitory factors against mRNAs of P. xylostella [16, 23]. Recently, a cys-motif protein of CpBV has been reported to be able to suppress the translation of host mRNAs [24]. CpBV-H4 is known to be able to regulate host gene expression in transcriptional level by an epigenetic mode [14, 16]. Thus, parasitism of C. plutellae can regulate host gene expression at both transcription level and translational level.

This study determined how many host genes of P. xylostella were regulated at transcriptional level by C. plutellae parasitism using RNA-Seq. In addition, this study determined the contribution of CpBV-H4 to host genes regulated by C. plutellae parasitism. DEG analysis of transcriptomes between parasitized and nonparasitized larvae at late stage determined host genes regulated by parasitism, in which 877 genes were up-regulated and 981 genes were down-regulated. These regulated host genes were predicted to have various functional categories including metabolism, development, immunity, signaling, and gene expression regulator. Venom proteins, ovarian proteins, teratocyte, and CpBV are known to be parasitic factors of C. plutellae [25, 26]. Venom and ovarian proteins produced from female wasp are delivered to parasitized host along with eggs to suppress host immunity or help PDV enter target cells [27, 28]. It has been suggested that these two factors play crucial roles during early parasitic stage [29]. However, the current study analyzed host gene expression at late parasitic stage. Thus, the altered host gene expression might be induced mainly by teratocyte and CpBV. It has been reported that P. xylostella larvae injected with teratocytes exhibit significant difference in expression pattern compared to untreated control larvae [26]. P. xylostella larvae injected with CpBV also can suppress host gene expression [30]. Especially, an SSH analysis has shown that CpBV-H4 alone can inhibit the expression levels of at least 115 host genes at transcription level [14]. Thus, the 1,858 host genes regulated by C. plutellae parasitism found in this study might have been influenced by both teratocyte and CpBV. Furthermore, this current study was focused on the single influence of CpBV-H4 on host gene regulation and how much it contributed to parasitism with respect to host gene regulation. Using the in vivo transient expression technique used to determine physiological functions of PDV genes in a previous study [31], CpBV-H4 was transiently expressed in nonparasitized larvae in this study. A truncated CpBV-H4 prepared by removing 38 amino acid residues at the N-terminal tail used in a previous study [11] was also expressed in the same aged larvae as a control. DEG analysis of these two transient expression groups resulted in 1,190 host genes specifically regulated in their expressions by CpBV-H4. Finally, the comparison of 1,858 genes regulated by parasitism and 1,190 genes regulated by CpBV-H4 showed that 302 host genes were overlapped in the two groups. Thus, 16.8% of host genes regulated by C. plutellae parasitism at late parasitic stage
Table 1. List of 81 up-regulated host genes after expressing viral gene CpBV-H4 in *P. xylostella*. Gene expression levels were compared between late third instar larvae parasitized (P7) or nonparasitized (NP5) by *C. plutellae* and expressed in fold change (FC) with respect to FPKM values. Gene identification (ID) followed the annotation of *P. xylostella* Genome Database (http://iae.fafu.edu.cn/DBM/index.php).

| Genes                                      | Gene ID  | FC (P7/NP5) | Functional categories |
|--------------------------------------------|----------|-------------|-----------------------|
| Larval cuticle protein LCP-30              | Px001339 | 63.3        | Development           |
| Larval cuticle protein LCP-30              | Px016045 | 63.3        | Development           |
| Death-associated small cytoplasmic leucine-rich protein | Px012314 | 40.4        | Development           |
| Urbain                                     | Px010057 | 29.6        | Signaling             |
| Retinol dehydrogenase                      | Px017386 | 25.4        | Metabolism            |
| Collagen alpha chain                       | Px013919 | 21.8        | Development           |
| Hypothetical                               | Px000870 | 16.6        | Unknown               |
| Neurofilament heavy polypeptide            | Px001530 | 15.8        | Signaling             |
| Hypothetical                               | Px010443 | 15.8        | Unknown               |
| Sulfortransferase                          | Px000114 | 15.1        | Metabolism            |
| Methyltransferase-like protein              | Px002962 | 13.4        | Development           |
| Hypothetical                               | Px015205 | 12.0        | Unknown               |
| Putative uncharacterized protein           | Px014022 | 7.6         | Unknown               |
| Hypothetical                               | Px009883 | 7.5         | Unknown               |
| Protein lethal essential for life          | Px003977 | 7.5         | Development           |
| Hypothetical                               | Px012012 | 7.1         | Unknown               |
| Hypothetical                               | Px011355 | 6.9         | Unknown               |
| Hypothetical                               | Px017694 | 6.5         | Unknown               |
| Protein PF14_0175                          | Px008075 | 5.4         | Signaling             |
| Neurofilament protein                      | Px005453 | 5.3         | Development           |
| Hypothetical                               | Px017104 | 5.2         | Signaling             |
| Hypothetical                               | Px003919 | 5.0         | Unknown               |
| Hypothetical                               | Px006831 | 4.7         | Unknown               |
| Alpha-tocopherol transfer protein-like     | Px004656 | 4.8         | Development           |
| Hypothetical                               | Px013516 | 4.4         | Development           |
| LIK1-like protein                          | Px008280 | 4.4         | Unknown               |
| Hypothetical                               | Px003203 | 4.3         | Unknown               |
| Hypothetical                               | Px015340 | 4.2         | Unknown               |
| Adenosine deaminase                        | Px004490 | 4.1         | Metabolism            |
| Gustatory receptor candidate               | Px008611 | 3.9         | Signaling             |
| Scavenger receptor class B member 1        | Px008150 | 3.8         | Signaling             |
| Protein lethal essential for life          | Px012769 | 3.8         | Development           |
| Glutathione S-transferase                  | Px006105 | 3.7         | Metabolism            |
| Hypothetical                               | Px002808 | 3.6         | Unknown               |
| Putative cuticle protein                   | Px016965 | 3.3         | Metabolism            |
| Serpin                                     | Px014258 | 3.4         | Development           |
| Hypothetical                               | Px010540 | 3.4         | Unknown               |
| Hypothetical                               | Px013167 | 3.4         | Unknown               |
| Fibrohexamer                               | Px012902 | 3.4         | Metabolism            |
| Hypothetical                               | Px001085 | 3.4         | Metabolism            |
| Hypothetical                               | Px016965 | 3.3         | Metabolism            |
| Hypothetical                               | Px012642 | 3.3         | Metabolism            |
| Hypothetical                               | Px010625 | 3.3         | Unknown               |
| Neurofilament heavy polypeptide            | Px016161 | 3.1         | Signaling             |
| Clavesin-1                                 | Px012514 | 3.1         | Development           |
| Hypothetical                               | Px008793 | 3.1         | Unknown               |

(Continued)
were induced by CpBV-H4 expression while the other 83.2% genes might have been regulated by other CpBV factors and teratocyte. Our current analysis supported the results of previous SSH analysis [14] because most (95%) down-regulated genes (115 genes) determined by SSH analysis were included in the down-regulated genes found in the current RNA-Seq analysis. In contrast, the remaining 888 host genes influenced by CpBV-H4 expression but not included in genes regulated by *C. plutellae* parasitism might be induced by unnatural effect of CpBV-H4 on host regulation such as excess amount (25 ng CpBV-H4 per larva) of gene dose compared to natural parasitism (< 1 ng per larva [32]) or the absence of interacting effect with other parasitic factors.

CpBV-H4 was mainly localized in 51 joining sites based on ChIP-Seq analysis. These joining sites were determined by overlapping sites based on the nearest genes between joining sites determined from parasitized larvae and larvae transiently expressing CpBV-H4. In-depth analysis of these joining sites showed that individual ChIP sites were unevenly distributed around target genes. However, this biased distribution did not influence the direction (up or down) of

Table 1. (Continued)

| Genes                          | Gene ID   | FC (P7/NP5) | Functional categories |
|-------------------------------|-----------|-------------|-----------------------|
| Hypothetical                  | Px002698  | 3.0         | Unknown               |
| Decaprenyl-diphosphate synthase| Px008402  | 3.0         | Metabolism            |
| Ejaculatory bulb-specific protein| Px009195  | 3.0         | Development           |
| Osiris                        | Px010922  | 2.9         | Development           |
| Putative uncharacterized protein| Px007085  | 2.9         | Unknown               |
| Neprilysin                    | Px017926  | 2.9         | Gene expression       |
| Probable nuclear hormone receptor| Px008400  | 2.9         | Signaling             |
| Hypothetical                  | Px004742  | 2.7         | Unknown               |
| Putative uncharacterized protein| Px009680  | 2.7         | Unknown               |
| Hypothetical                  | Px001678  | 2.7         | Unknown               |
| Proteasome subunit alpha type-2| Px010913  | 2.7         | Development           |
| Hypothetical                  | Px001472  | 2.7         | Unknown               |
| Hypothetical                  | Px012390  | 2.7         | Unknown               |
| Helix-loop-helix protein Delilah| Px005488  | 2.6         | Development           |
| Putative cuticle protein CPH36| Px015917  | 2.5         | Development           |
| Clavin-1                      | Px004516  | 2.5         | Development           |
| Hypothetical                  | Px003928  | 2.5         | Unknown               |
| Probable dolichol-phosphate mannosyltransferase | Px012743 | 2.4         | Metabolism            |
| Hypothetical                  | Px006833  | 2.4         | Unknown               |
| Hypothetical                  | Px008161  | 2.4         | Unknown               |
| Hypothetical                  | Px007157  | 2.4         | Unknown               |
| Hypothetical                  | Px006388  | 2.3         | Unknown               |
| Hypothetical                  | Px008548  | 2.3         | Unknown               |
| Putative odorant-binding protein A10| Px014885  | 2.3         | Signaling             |
| Hypothetical                  | Px012010  | 2.2         | Unknown               |
| Putative inorganic phosphate cotransporter | Px013082 | 2.2         | Metabolism            |
| Alpha-tocopherol transfer protein-like | Px001573 | 2.2         | Development           |
| Carboxypeptidase B             | Px005373  | 2.2         | Development           |
| Hypothetical                  | Px010142  | 2.2         | Unknown               |
| Endochitinase                 | Px008062  | 2.2         | Development           |
| Hypothetical                  | Px003497  | 2.2         | Unknown               |

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Table 2. List of 221 host genes down-regulated by the expression of a viral gene CpBV-H4 in *P. xylostella*. Gene expression levels were compared between late third instar larvae parasitized (P7) or nonparasitized (NP5) by *C. plutellae* and expressed in fold change (FC) with respect to FPKM values. Gene identification (ID) followed the annotation of *P. xylostella* Genome Database (http://iae.fafu.edu.cn/DBM/index.php). Negative value indicates decreased expression of P7 compared to NP5.

| Genes                          | Gene ID     | FC (P7/NP5) | Functional categories |
|-------------------------------|-------------|-------------|-----------------------|
| Lipase                        | Px011477    | -2.0        | Metabolism            |
| Cytosolic β-glucosidase       | Px010022    | -2.0        | Metabolism            |
| Trypsin                       | Px007677    | -2.0        | Development           |
| Trypsin                       | Px006570    | -2.0        | Development           |
| Superoxide dismutase [Cu-Zn]  | Px001161    | -2.1        | Signaling             |
| Chitin deacetylase            | Px001430    | -2.1        | Development           |
| Esterase                      | Px011443    | -2.1        | Metabolism            |
| Mitochondrial 2-oxoglutarate  | Px009263    | -2.1        | Metabolism            |
| Sodium/potassium/calcium exchanger | Px002564 | -2.1        | Metabolism            |
| Trypsin                       | Px005242    | -2.1        | Development           |
| Cytochrome b5-related protein | Px010558    | -2.1        | Development           |
| Beta-ureidopropionase         | Px017763    | -2.1        | Signaling             |
| Glutathione S-transferase     | Px006481    | -2.1        | Metabolism            |
| GTP: AMP phosphotransferase, mitochondrial | Px012467 | -2.1 | Metabolism |
| Cytochrome b-c1 complex       | Px011320    | -2.1        | Metabolism            |
| Inositol oxygenase            | Px016791    | -2.1        | Signaling             |
| Trypsin                       | Px007619    | -2.1        | Development           |
| Trypsin                       | Px016058    | -2.1        | Development           |
| Aminoaclase                   | Px001371    | -2.1        | Metabolism            |
| Protein henna similar to PH4H_DROME | Px004945 | -2.1 | Signaling |
| Labial Similar to D6W945_TRICA | Px016748 | -2.2 | Signaling |
| Hexokinase                    | Px010425    | -2.2        | Metabolism            |
| General odorant-binding protein| Px004433    | -2.2        | Signaling             |
| Hexokinase                    | Px003540    | -2.2        | Metabolism            |
| Serine protease               | Px016741    | -2.2        | Immune                |
| Lipase                        | Px012694    | -2.2        | Metabolism            |
| Monocarboxylate transporter   | Px006257    | -2.2        | Signaling             |
| Aminopeptidase N              | Px001708    | -2.2        | Metabolism            |
| Peritrophin type-A domain protein | Px004696 | -2.2 | Metabolism |
| Chymotrypsin                  | Px005554    | -2.2        | Development           |
| Hypothetical                  | Px014003    | -2.2        | Unknown               |
| 1-acyl-sn-glycerol-3-phosphate acyltransferase-α | Px017143 | -2.2 | Metabolism |
| Collagenase                   | Px005340    | -2.2        | Development           |
| Acyl-CoA oxidase              | Px017102    | -2.2        | Metabolism            |
| SWI/SNF complex subunit SMARCC2 | Px016803 | .22 | Immune |
| Hypothetical                  | Px014974    | -2.3        | Unknown               |
| Lipase                        | Px012695    | -2.3        | Metabolism            |
| Uridine phosphorylase         | Px009019    | -2.3        | Metabolism            |
| Luciferin 4-monoxygenase      | Px010831    | -2.3        | Metabolism            |
| Arylsulfatase B               | Px010550    | -2.3        | Development           |
| 4-coumarate—CoA ligase        | Px011113    | -2.3        | Metabolism            |
| 3-oxoacyl-[acyl-carrier-protein] reductase FabG | Px018005 | -2.3 | Metabolism |
| Cytochrome b561 domain-containing protein | Px008446 | -2.3 | Development |
| Protein 5NUC                  | Px012027    | -2.3        | Signaling             |
| Sensory neuron membrane protein| Px013824    | -2.4        | Signaling             |

(Continued)
### Table 2. (Continued)

| Genes                                             | Gene ID      | FC (P7/NP5) | Functional categories |
|----------------------------------------------------|--------------|-------------|-----------------------|
| Collagenase                                        | Px012582     | -2.4        | Development           |
| Chymotrypsin BI                                   | Px007900     | -2.4        | Development           |
| Aminoacylase-1A                                   | Px008322     | -2.4        | Metabolism            |
| Long-chain-fatty-acid—CoA ligase ACSBG2            | Px008837     | -2.4        | Metabolism            |
| Membrane alanyl aminopeptidase                    | Px008278     | -2.4        | Signaling             |
| Pancreatic triacylglycerol lipase                 | Px002296     | -2.4        | Metabolism            |
| Peritrophin type-A domain protein 2               | Px007019     | -2.4        | Metabolism            |
| Chymotrypsin-like elastase family member 2B       | Px011888     | -2.4        | Development           |
| Protein ETHE1, mitochondrial                      | Px005329     | -2.5        | Metabolism            |
| Hexokinase                                         | Px001060     | -2.5        | Metabolism            |
| Putative inorganic phosphate cotransporter       | Px014024     | -2.5        | Metabolism            |
| Zinc carboxypeptidase A                           | Px000994     | -2.5        | Metabolism            |
| Glucose dehydrogenase                             | Px011825     | -2.5        | Metabolism            |
| Solute carrier family 22 member 21                | Px017195     | -2.5        | Signaling             |
| Ribosome-binding protein                          | Px009784     | -2.5        | Gene expression       |
| Myrosinase                                         | Px002081     | -2.5        | Metabolism            |
| Proton-coupled amino acid transporter             | Px007390     | -2.5        | Signaling             |
| Probable E3 ubiquitin-protein ligase sinah        | Px008209     | -2.5        | Signaling             |
| Trypsin, alkaline B                               | Px009284     | -2.5        | Signaling             |
| Transferrin                                        | Px011514     | -2.6        | Signaling             |
| Retinoid-inducible serine carboxypeptidase        | Px000211     | -2.6        | Development           |
| Membrane alanyl aminopeptidase                    | Px003754     | -2.6        | Signaling             |
| Carboxypeptidase O                                | Px000991     | -2.6        | Development           |
| Phosphotriesterase-related protein                | Px009117     | -2.6        | Metabolism            |
| Trypsin                                           | Px016057     | -2.6        | Development           |
| Hypothetical                                      | Px010793     | -2.6        | Unknown               |
| Hypothetical                                      | Px008726     | -2.7        | Unknown               |
| Facilitated trehalose transporter                 | Px009445     | -2.7        | Development           |
| Glutathione S-transferase                         | Px010078     | -2.7        | Metabolism            |
| Putative inorganic phosphate cotransporter       | Px013678     | -2.7        | Development           |
| Sedoheptulokinase                                 | Px007641     | -2.7        | Development           |
| Acyl-CoA synthetase family member                 | Px016733     | -2.7        | Metabolism            |
| Hypothetical                                      | Px004934     | -2.7        | Unknown               |
| Prostaglandin reductase                           | Px009370     | -2.8        | Immune                |
| Membrane alanyl aminopeptidase                    | Px008277     | -2.8        | Metabolism            |
| Hypothetical                                      | Px013342     | -2.8        | Unknown               |
| Plasma glutamate carboxypeptidase                 | Px009761     | -2.8        | Metabolism            |
| Transferrin                                        | Px012137     | -2.8        | Signaling             |
| 2-Oxoisovalerate dehydrogenase subunit alpha      | Px006816     | -2.8        | Metabolism            |
| Glyoxylate reductase/hydroxy.pyruvate reductase   | Px004525     | -2.8        | Metabolism            |
| Inactive dipeptidyl peptidase                     | Px013212     | -2.8        | Metabolism            |
| Facilitated trehalose transporter                 | Px012298     | -2.8        | Development           |
| Trypsin                                           | Px001804     | -2.9        | Development           |
| Monocarboxylate transporter                       | Px005246     | -2.9        | Signaling             |
| Sucrose-6-phosphate hydrolase                     | Px001761     | -2.9        | Metabolism            |
| Lipase                                            | Px005804     | -2.9        | Metabolism            |
| Galactokinase                                     | Px005810     | -2.9        | Metabolism            |

(Continued)
| Genes                                      | Gene ID       | FC (P7/NP5) | Functional categories |
|--------------------------------------------|---------------|-------------|-----------------------|
| Multiple C2 and transmembrane domain       | Px005022      | -2.9        | Signaling             |
| C-1-tetrahydrofolate synthase, cytoplasmic | Px003316      | -2.9        | Development           |
| Lysine-specific demethylase NO66           | Px009382      | -3.0        | Immune                |
| Gamma-glutamyl hydrolase A                 | Px011489      | -3.0        | Metabolism            |
| Cytochrome P450 6B6                        | Px005902      | -3.0        | Development           |
| Collagenase                                | Px007902      | -3.1        | Development           |
| Midgut protein Lstil09                     | Px014861      | -3.1        | Development           |
| Hypothetical                               | Px005937      | -3.1        | Unknown               |
| Arylphorin subunit alpha                   | Px007028      | -3.1        | Gene expression       |
| Trypsin                                    | Px005241      | -3.1        | Development           |
| Membrane alanyl aminopeptidase             | Px003755      | -3.1        | Signaling             |
| GH11122                                    | Px012786      | -3.1        | Development           |
| Luciferin 4-monoxygenase                   | Px016655      | -3.1        | Metabolism            |
| Hypothetical                               | Px014245      | -3.1        | Unknown               |
| Apolipoporphins                            | Px015730      | -3.1        | Development           |
| Ribose-phosphate pyrophosphokinase         | Px011035      | -3.1        | Metabolism            |
| Chymotrypsin-C                             | Px007676      | -3.2        | Development           |
| Xanthine dehydrogenase                     | Px002720      | -3.3        | Metabolism            |
| Facilitated trehalose transporter          | Px004014      | -3.3        | Development           |
| Myrosinase                                 | Px009427      | -3.3        | Metabolism            |
| Retinol dehydrogenase                      | Px000793      | -3.3        | Metabolism            |
| ACYI004563 protein                         | Px016078      | -3.4        | Signaling             |
| Probable peroxisomal acyl-coenzyme A oxidase 1 | Px001531 | -3.4 | Metabolism             |
| Collagenase                                | Px005342      | -3.4        | Development           |
| Hypothetical                               | Px012051      | -3.4        | Unknown               |
| Trypsin                                    | Px007621      | -3.4        | Development           |
| Transmembrane inner ear expressed protein  | Px007550      | -3.4        | Signaling             |
| Probable dihydropyrimidine dehyd. [NADP+] | Px014464      | -3.5        | Metabolism            |
| Trypsin                                    | Px015277      | -3.5        | Development           |
| L-ascorbate oxidase                        | Px001443      | -3.5        | Metabolism            |
| 3-oxoacyl-[acyl-carrier-protein] reductase FabG | Px016235 | -3.5 | Metabolism             |
| Acetylcholinesterase                       | Px011049      | -3.5        | Development           |
| Estradiol 17-β-dehydrogenase               | Px015642      | -3.6        | Development           |
| galactose-1-phosphate uridylyltransferase  | Px004395      | -3.6        | Development           |
| Dihydropyrimidine dehydrogenase [NADP+]    | Px003428      | -3.6        | Development           |
| Esterase                                   | Px006430      | -3.6        | Development           |
| Angiotensin-converting enzyme              | Px012643      | -3.6        | Development           |
| Aminomethyltransferase, mitochondrial      | Px011054      | -3.7        | Metabolism            |
| Ecdysteroid UDP-glucosyltransferase        | Px000872      | -3.7        | Development           |
| Sodium- and chloride-dep. glycine transporter | Px009514 | -3.8 | Signaling             |
| Dihydropyrimidine dehydrogenase [NADP+]    | Px000264      | -3.8        | Development           |
| Hypothetical                               | Px004235      | -3.8        | Unknown               |
| Angiotensin-converting enzyme              | Px001633      | -3.8        | Signaling             |
| Hypothetical                               | Px001337      | -3.9        | Unknown               |
| Hypothetical                               | Px010854      | -3.9        | Unknown               |
| Esterase                                   | Px002735      | -3.9        | Metabolism            |
| Phosphoenolpyruvate carboxykinase [GTP]    | Px015376      | -3.9        | Metabolism            |
Table 2. (Continued)

| Genes                                           | Gene ID    | FC (P7/NP5) | Functional categories |
|-------------------------------------------------|------------|-------------|-----------------------|
| Mitochondrial ornithine transporter 1           | Px007072   | -3.9        | Metabolism            |
| Luciferin 4-monoxygenase                        | Px003138   | -3.9        | Metabolism            |
| Ecdysteroid UDP-glucosyltransferase             | Px004854   | -3.9        | Development           |
| Trypsin                                         | Px006572   | -4.0        | Development           |
| Lipase                                          | Px011610   | -4.0        | Development           |
| Peritrophic matrix insect intestinal mucin      | Px007895   | -4.1        | Development           |
| Glucosidase KIAA1161                           | Px002046   | -4.1        | Metabolism            |
| Acetylcholinesterase                           | Px00089    | -4.1        | Development           |
| Luciferin 4-monoxygenase                        | Px012806   | -4.1        | Development           |
| Estradiol 17-β-dehydrogenase                    | Px008771   | -4.1        | Development           |
| Cytochrome P450 6B5                            | Px013454   | -4.1        | Signaling             |
| Trypsin                                         | Px002864   | -4.2        | Development           |
| Bifunctional purine biosynthesis protein        | Px011885   | -4.3        | Metabolism            |
| Carboxypeptidase                                | Px000996   | -4.3        | Development           |
| Trypsin                                         | Px000107   | -4.4        | Development           |
| Phosphoserine phosphatase                      | Px000750   | -4.4        | Development           |
| Hypothetical                                    | Px012836   | -4.5        | Unknown               |
| C-1-tetrahydrofolate synthase, cytoplasmic     | Px007305   | -4.5        | Metabolism            |
| Isovaleryl-CoA dehydrogenase, mitochondrial     | Px014703   | -4.6        | Metabolism            |
| Pancreatic lipase-related protein               | Px005193   | -4.7        | Development           |
| Sorbitol dehydrogenase                         | Px000215   | -4.8        | Metabolism            |
| Probable maltase                                | Px003486   | -4.8        | Development           |
| Multifunctional protein ADE2                    | Px011706   | -4.9        | Signaling             |
| Phosphoserine phosphatase                      | Px007835   | -4.9        | Development           |
| Acetylcholinesterase                           | Px009940   | -4.9        | Development           |
| Trypsin                                         | Px012568   | -5.0        | Development           |
| Hypothetical                                    | Px006985   | -5.2        | Unknown               |
| Sorbitol dehydrogenase                         | Px004996   | -5.3        | Metabolism            |
| Juvenile hormone esterase                       | Px012592   | -5.3        | Development           |
| Synaptic vesicle glycoprotein                   | Px001753   | -5.3        | Signaling             |
| Hypothetical                                    | Px004933   | -5.4        | Unknown               |
| Myrosinase 1                                    | Px006942   | -5.4        | Metabolism            |
| Pancreatic triacylglycerol lipase               | Px000644   | -5.5        | Development           |
| Esterase                                        | Px011756   | -5.6        | Development           |
| Ecdysteroid-regulated protein                   | Px00634    | -5.6        | Development           |
| Glutaryl-CoA dehydrogenase, mitochondrial       | Px011286   | -5.7        | Development           |
| Peritrophin-1                                   | Px010130   | -5.8        | Signaling             |
| Elongation of very long chain fatty acids protein| Px013748   | -5.9        | Development           |
| Larval cuticle protein                          | Px003260   | -6.0        | Development           |
| Peritrophic matrix insect intestinal mucin      | Px007897   | -6.1        | Development           |
| Ecdysteroid-regulated protein                   | Px011111   | -6.1        | Development           |
| Antennal esterase                               | Px011755   | -6.1        | Development           |
| Phosphoenolpyruvate carboxykinase               | Px010887   | -6.1        | Metabolism            |
| Pancreatic triacylglycerol lipase               | Px012011   | -6.2        | Development           |
| Hypothetical                                    | Px013960   | -6.4        | Unknown               |
| Glycine N-methyltransferase                     | Px003291   | -6.4        | Immune                |
| Sialin                                          | Px004317   | -6.5        | Development           |
gene regulation. Thus, target genes can be up- or down- regulated irrespective of biased distribution of CpBV-H4 on upstream, gene body, or downstream locations. A previous study [17] pointed out that CpBV-H4s are localized on promoter regions of inducible target genes which might be highly involved in chromatin remodeling. However, the current ChIP-Seq analysis showed that CpBV-H4 could be integrated into gene body or downstream as well as upstream possessing promoter regions.

Physical mapping of joining sites of CpBV-H4 showed that CpBV-H4s were localized mainly into a small number of chromosomes. In contrast, host genes regulated by CpBV-H4

| Genes                                      | Gene ID   | FC (P7/NP5) | Functional categories |
|--------------------------------------------|-----------|-------------|-----------------------|
| Myrosinase 1                               | Px006054  | -6.6        | Development           |
| Oxidoreductase ucpA                        | Px002029  | -6.7        | Development           |
| Hypothetical                               | Px001077  | -6.7        | Unknown               |
| Prostaglandin reductase                    | Px006403  | -6.9        | Immune                |
| Trypsin                                    | Px010386  | -7.0        | Development           |
| Choline dehydrogenase, mitochondrial       | Px002926  | -7.1        | Metabolism            |
| Facilitated trehalose transporter          | Px002482  | -7.1        | Development           |
| Putative acyl-CoA-binding protein          | Px000524  | -7.2        | Metabolism            |
| Sucrose-6-phosphate hydrolase              | Px004218  | -7.2        | Development           |
| Luciferin 4-monooxygenase                  | Px016492  | -7.3        | Metabolism            |
| Phosphoenolpyruvate carboxykinase          | Px015377  | -7.3        | Development           |
| Zinc carboxypeptidase A                    | Px015831  | -7.4        | Metabolism            |
| Putative acyl-CoA-binding protein          | Px001605  | -7.4        | Metabolism            |
| Adenylate kinase isoenzyme 1               | Px018022  | -7.4        | Metabolism            |
| Hypothetical                               | Px012853  | -7.5        | Unknown               |
| Carboxypeptidase B                         | Px010017  | -7.5        | Development           |
| Probable D-xylulose reductase A            | Px013954  | -7.7        | Development           |
| Collagenase                                | Px013665  | -7.7        | Development           |
| Alpha-amylase 4N                           | Px000395  | -8.0        | Metabolism            |
| Larval cuticle protein 16/17               | Px003261  | -8.0        | Development           |
| Esterase                                   | Px011757  | -8.5        | Development           |
| Hypothetical                               | Px006547  | -8.5        | Unknown               |
| Carboxypeptidase                           | Px015830  | -8.7        | Development           |
| Collagenase                                | Px005341  | -8.8        | Development           |
| Glutathione S-transferase                  | Px006106  | -8.9        | Signaling             |
| Lactase-phlorizin hydrolase                | Px005277  | -9.1        | Metabolism            |
| Fibrohexamer                               | Px001076  | -10.2       | Signaling             |
| Glucose dehydrogenase [acceptor]          | Px008505  | -10.2       | Development           |
| Putative acyl-CoA-binding protein          | Px017039  | -11.8       | Metabolism            |
| Hypothetical                               | Px013308  | -11.9       | Unknown               |
| α-amylase                                  | Px000394  | -13.2       | Metabolism            |
| Chitin binding PM protein                  | Px001431  | -13.8       | Development           |
| Trypsin                                    | Px012570  | -14.6       | Development           |
| Lactase-phlorizin hydrolase                | Px011160  | -15.2       | Development           |
| Repetitive proline-rich cell wall protein  | Px010466  | -16.7       | Metabolism            |
| Vesicular glutamate transporter            | Px014303  | -21.5       | Signaling             |
| Hypothetical                               | Px016820  | -153.5      | Unknown               |

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were not close to CpBV-H4 joining sites. Some target genes controlled by CpBV-H4 were localized on chromosomes in which CpBV-H4 was not integrated. This suggests that genes regulated by CpBV-H4 might be able to regulate subsequent target genes by acting as transcriptional regulators. Indeed, target genes regulated by CpBV-H4 included transcription regulators involved in gene expression. For example, the expression levels of Px-KDM and Px-
Table 3. List of 51 common ChIP targets of CpBV-H4 (vH4) on *P. xylostella* genome from analysis of chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq).

| ChIP targets   | Location on *P. xylostella* genome | Distance (bp) to nearest CDS | Nearest CDS (GenBank accession #) | FC (P7/NP5) | Functional categories |
|----------------|------------------------------------|------------------------------|-----------------------------------|-------------|-----------------------|
| vH4-ChIP-1     | ChrLG-1 (267416–267973)            | -557                         | Hypothetical (Px013825)           | 5.0         | Unknown               |
| vH4-ChIP-2     | ChrLG-5 (276105–276260)            | 155                          | Hypothetical (Px008241)           | 4.2         | Unknown               |
| vH4-ChIP-3     | ChrLG-5 (215111–219713)            | -602                         | Cleft lip and palate transmembrane protein 1-like protein (Px008237) | -10.3       | Signaling             |
| vH4-ChIP-4     | ChrLG-5 (316488–318790)            | -473                         | Centrosomal protein of 97 kDa (Px004309) | -3.0        | Development           |
| vH4-ChIP-5     | ChrLG-6 (478831–486427)            | 596                          | Uncharacterized protein KIAA0090 homolog (Px003642) | 10.8        | Unknown               |
| vH4-ChIP-6     | ChrLG-7 (156448–162473)            | -447                         | Zinc finger protein (Px011497)    | 12.2        | Signaling             |
| vH4-ChIP-7     | ChrLG-12 (239288–249465)           | 472                          | Mitochondrial glutamate carrier (Px008056) | -14.2       | Metabolism            |
| vH4-ChIP-8     | ChrLG-12 (59259–80699)             | 683                          | GJ16239 (Px008047)               | 8.5         | Metabolism            |
| vH4-ChIP-9     | ChrLG-14 (271091–271922)           | 831                          | Putative uncharacterized protein (Px010174) | -10.1       | Unknown               |
| vH4-ChIP-10    | ChrLG-17 (112167–1126344)          | -526                         | Protein penguin (Px009354)        | 8.0         | Development           |
| vH4-ChIP-11    | ChrLG-21 (882018–882712)           | 694                          | HIG1 domain family member 2A (Px002719) | -4.2        | Gene expression       |
| vH4-ChIP-12    | ChrLG-22 (883663–922749)           | 469                          | Polycomb protein edd-A (Px002998) | 18.2        | Development           |
| vH4-ChIP-13    | ChrLG-22 (1306408–1317112)         | -704                         | Uncharacterized protein KIAA1370 (Px003709) | -2.3        | Unknown               |
| vH4-ChIP-14    | ChrLG-24 (470439–478675)           | 236                          | Endothelial zinc finger protein induced by TNF alpha (Px006727) | 5.0         | Development           |
| vH4-ChIP-15    | ChrLG-26 (50771–68538)             | 767                          | Zinc finger protein (Px010512)    | 25.1        | Signaling             |
| vH4-ChIP-16    | ChrLG-UN (16658–37064)             | 406                          | Leucine-rich repeat-containing protein (Px016400) | -7.5        | Metabolism            |
| vH4-ChIP-17    | ChrLG-UN (107271–120081)           | 810                          | Aldehyde dehydrogenase family 1 member L1 (Px013663) | -45.6       | Metabolism            |
| vH4-ChIP-18    | ChrLG-UN (204710–223658)           | 684                          | S1D1 transmembrane family member 1 (Px017204) | -40.4       | Metabolism            |
| vH4-ChIP-19    | ChrLG-UN (75155–82548)             | -582                         | GTPase-activating protein (Px014828) | -25.0       | Metabolism            |
| vH4-ChIP-20    | ChrLG-UN (12872–14358)             | 829                          | Vacular protein sorting-associated protein (Px001552) | 24.0        | Metabolism            |
| vH4-ChIP-21    | ChrLG-UN (224911–226167)           | -738                         | α-(1,3)-fucosyltransferase (Px005358) | 18.2        | Metabolism            |
| vH4-ChIP-22    | ChrLG-UN (3739–5950)               | 621                          | Mitochondrial intermediate peptidase (Px002154) | 18.2        | Metabolism            |
| vH4-ChIP-23    | ChrLG-UN (1709593–1710105)         | 512                          | Hypothetical (Px005915)           | -16.9       | Unknown               |
| vH4-ChIP-24    | ChrLG-UN (696255–714576)           | 944                          | Dual serine/threonine and tyrosine protein kinase (Px006800) | -16.0       | Immune                |
| vH4-ChIP-25    | ChrLG-UN (44835–47452)             | -654                         | Hypothetical (Px005285)           | -16         | Unknown               |
| vH4-ChIP-26    | ChrLG-UN (4769–6486)               | 26                           | Putative transposase ykgN (Px017967) | -14.2       | Gene expression       |
| vH4-ChIP-27    | ChrLG-UN (88864–107215)            | 828                          | Hypothetical (Px013605)           | 14.2        | Unknown               |
| vH4-ChIP-28    | ChrLG-UN (172060–172677)           | -617                         | Hypothetical (Px005697)           | -13.7       | Unknown               |
| vH4-ChIP-29    | ChrLG-UN (998583–1039637)          | 125                          | Nucleolar MIF4G domain-containing protein 1 homolog (Px014314) | -13.4       | Gene expression       |
| vH4-ChIP-30    | ChrLG-UN (78554–80038)             | -832                         | MORN repeat-containing protein (Px014423) | 13.1        | Gene expression       |
| vH4-ChIP-31    | ChrLG-UN (186009–186577)           | 478                          | Hypothetical (Px012337)           | 13.0        | Unknown               |
| vH4-ChIP-32    | ChrLG-UN (475306–475995)           | -689                         | A7S037_N EMVE; Predicted protein (Px004214) | -12.3       | Gene expression       |
| vH4-ChIP-33    | ChrLG-UN (13224–59610)             | 386                          | Rapamycin-insensitive companion of m-Tor (Px015368) | 12.1        | Signaling             |
| vH4-ChIP-34    | ChrLG-UN (112006–130292)           | 286                          | Exostosin-2 (Px013363)            | 12.0        | Gene expression       |
| vH4-ChIP-35    | ChrLG-UN (16056–17294)             | 755                          | Facilitated trehalose transporter (Px016880) | -10.4       | Development           |
| vH4-ChIP-36    | ChrLG-UN (1622315–1627191)         | 876                          | Regulator of G-protein signaling (Px016347) | -10.3       | Signaling             |

(Continued)
SWI/SNF were suppressed by CpBV-H4 in both SSH and RNA-Seq analyses. KDM and SWI/SNF are known to be able to regulate other gene transcription in an epigenetic mode [33, 34]. Furthermore, these two genes are required for immunity and development of *P. xylostella* [14]. Thus, CpBV-H4 can influence host gene expression by directly binding to chromatin around target genes or by indirectly influencing host gene expression by regulating transcriptional regulators.

This study found that 302 host genes were regulated by CpBV-H4 with joining sites near 51 target genes. Furthermore, this study explained a significant contribution (16.8%) of CpBV-H4 on host gene regulation induced by *C. plutellae* parasitism because teratocytes and other 156 CpBV genes might play crucial roles in altering host gene expression. Similar viral histone H4s have been found in other *Cotesia*-associated BVs [35]. Thus, viral histone H4 might be a main parasitic factor in *Cotesia*-associated parasitism.

**Materials and methods**

**Insects and parasitization**

*P. xylostella* larvae were reared with cabbage leaves at 25 ± 1 °C with photoperiod of 16:8 h (L:D). Adults were fed 10% sucrose. Late first instar larvae were parasitized by *C. plutellae* at 1:2 (wasp: host) density for 24 h under the rearing condition. Parasitized larvae were fed cabbage leaves. Parasitized larvae lived for 8 days (P1–P8). They died after the emergence of fully matured wasp larva which shortly formed cocoons for pupal development. In contrast, Non-parasitized larvae at the age corresponding to P larvae at parasitization lived 6 days (NP1–NP6) at 25 °C and pupated. Thus, P1 was an equivalent age to NP1 while P7 was an equivalent age to P7. Wasp cocoons were collected and kept at 25 °C for adult tissue development. After emergence, adult wasps were allowed to mate for 24 h. They were then used for parasitization.

| Table 3. (Continued) |
|----------------------|
| **ChIP targets** | **Location on *P. xylostella* genome** | **Distance (bp) to nearest CDS** | **Nearest CDS (GenBank accession #)** | **FC (P7/NP5)**<sup>b</sup> | **Functional categories** |
| vH4-ChIP-37 | ChrLG-UN (1345193–1351114) | -921 | Ras-related protein RabJ (Px006237) | -10.1 | Signaling |
| vH4-ChIP-38 | ChrLG-UN (600031–600513) | -482 | Probable serine hydrolase (Px008214) | 8.6 | Metabolism |
| vH4-ChIP-39 | ChrLG-UN (89183–118306) | -987 | Protein PAT1 homolog (Px010075) | -8.4 | Gene expression |
| vH4-ChIP-40 | ChrLG-UN (273969–274525) | 556 | Hypothetical (Px007509) | 7.45 | Unknown |
| vH4-ChIP-41 | ChrLG-UN (108141–109100) | 959 | Hypothetical (Px009739) | -6.8 | Unknown |
| vH4-ChIP-42 | ChrLG-UN (2292630–2293702) | -261 | Larval cuticle protein LCP-30 (Px003250) | -6.6 | Development |
| vH4-ChIP-43 | ChrLG-UN (2294845–2297228) | 118 | Putative cuticle protein (Px003251) | -6.6 | Development |
| vH4-ChIP-44 | ChrLG-UN (448023–448313) | 290 | Hypothetical (Px004665) | 6.5 | Unknown |
| vH4-ChIP-45 | ChrLG-UN (709982–756563) | 122 | Protein dpy-19 homolog (Px002457) | 6.4 | Metabolism |
| vH4-ChIP-46 | ChrLG-UN (1513940–1538960) | 543 | UBX domain-containing protein 4 (Px006242) | 5.8 | Gene expression |
| vH4-ChIP-47 | ChrLG-UN (192843–194073) | -891 | Hypothetical (Px012789) | -10.7 | Unknown |
| vH4-ChIP-48 | ChrLG-UN (390910–391918) | 312 | Hypothetical (Px003836) | -7.7 | Unknown |
| vH4-ChIP-49 | ChrLG-UN (417484–423132) | 648 | Hypothetical (Px004564) | -6.5 | Unknown |
| vH4-ChIP-50 | ChrLG-UN (288781–289959) | 669 | Probable 39S ribosomal protein L45 (Px005650) | 6.3 | Gene expression |
| vH4-ChIP-51 | ChrLG-UN (1681979–1682541) | -562 | Trypsin (Px002590) | -4.9 | Development |

<sup>a</sup> + and - represent upstream and downstream, respectively, from the nearest coding DNA sequence (CDS)

<sup>b</sup> Fold change (FC) in FPKM values

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Fig 4. DNA sequence characters of CpBV-H4 joining sites on *P. xylostella* genome. (A) Occurrence of two nucleotide repeat motifs among ChIP targets (480 sites assessed) against chromatin extracted from host larvae parasitized by *C. plutellae*. (B) Expression profiles of genes close to CpBV-H4 joining sites containing repeat motifs. Up/down-regulated genes are defined by at least two-fold change in FPKM values.

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Table 4. Characters of DNA sequences in 480 ChIP targets of CpBV-H4 against chromatins derived from *P. xylostella* parasitized by *C. plutellae*. ChIPs are localized at upstream (UP), downstream (DOWN), or gene body (GB) with respect to the nearest coding DNA sequence (CDS).

| ChIP targets | Location to CDS | ChIP targets | Location to CDS | ChIP targets | Location to CDS |
|--------------|-----------------|--------------|-----------------|--------------|-----------------|
| GT-repeating  |                 |              |                 |              |                 |
| SC_01 (1529579–1529733) | UP | SC_13 (305106–305260) | DOWN | SC_75 (666947–667101) | DOWN |
| SC_04 (643998–644152) | UP | SC_141 (88466–88620) | UP | SC_83 (244226–244380) | DOWN |
| SC_07 (317692–317846) | UP | SC_143 (403725–403879) | UP | SC_90 (137739–137893) | UP |
| SC_11 (403487–403641) | DOWN | SC_143 (330068–330222) | UP | SC_98 (152754–152908) | DOWN |
| SC_12 (1683234–1683388) | GB | SC_147 (914126–914280) | GB | SC_99 (556050–556204) | UP |
| SC_18 (443738–443892) | GB | SC_159 (481456–481610) | GB | SC_106 (43556–43710) | UP |
| SC_19 (1411864–1412018) | GB | SC_170 (316051–316163) | UP | SC_182 (181374–181528) | UP |
| SC_20 (846120–846274) | GB | SC_196 (47489–47643) | DOWN | SC_200 (48915–49069) | UP |
| SC_23 (890357–890511) | UP | SC_202 (288112–288266) | UP | SC_628 (73207–73361) | GB |
| SC_28 (77042–77196) | UP | SC_227 (95085–95239) | UP | SC_666 (69847–70001) | UP |
| SC_29 (114735–1141889) | DOWN | SC_236 (466266–466378) | UP | SC_730 (8408–8562) | GB |
| SC_32 (1125668–1125822) | GB | SC_275 (311126–311280) | UP | SC_108 (230141–230295) | UP |
| SC_33 (1120935–1121091) | DOWN | SC_287 (241760–241914) | UP | SC_120 (600098–600252) | GB |
| SC_35 (1079097–1079251) | GB | SC_294 (221145–221299) | DOWN | SC_120 (764303–764547) | GB |
| SC_36 (91667–91821) | GB | SC_300 (206688–206842) | GB | SC_809 (35039–35193) | UP |
| SC_37 (245401–245513) | GB | SC_305 (262500–262654) | GB | SC_960 (17952–18051) | GB |
| SC_38 (388099–388253) | GB | SC_310 (249522–249666) | GB | SC_130 (736354–736508) | GB |
| SC_39 (1307832–1307986) | GB | SC_315 (65035–65189) | UP | SC_131 (9571–9654) | UP |

(Continued)
Transient expression of CpBV-H4 in *P. xylostella* larvae

A gene encoding CpBV-H4 containing N-terminal tail (vH4) and truncated CpBV-H4 (vH4T) after deleting N-terminal tail was cloned into a pIB vector [17]. The recombinant plasmid (250 ng/μL) was mixed in an equal volume of Metafectene PRO transfection reagent (Biontex, Planegg, Germany) and incubated for 20 min at room temperature to allow the formation of DNA-lipid complex. A total of 100 nL of this DNA-lipid complex was injected into the hemocoel of NP3 larvae at a rate 10 nL/sec using microsyringe pump controller (PV830 Pneumatic Pico Pump, World Precision Instruments, Sarasota, FL, USA) under a microscope (Olympus S730, Tokyo, Japan). At 48 h post-infection, transient expression of vH4 was confirmed by RT-PCR using forward primer

5′-GGATCCATGGCTGATCATCCTCTAAAGG-3′

and reverse primer

5′-GAATTCTCAACCTCCATAACCATAGTC-3′. The expression of vH4T was...
Fig 5. Physical mapping of 51 CpBV-H4 joining sites on *P. xylostella* chromosomes. A total of 29 chromosomes are presented in linkage groups ('LGs'). Of the 51 CpBV-H4 joining sites, 15 sites (down arrows) are denoted on 11 LGs while the other 36 joining sites are localized in uncharacterized LG ('LGUN'). Differentially expressed genes (DEGs) after *CpBV-H4* expression are denoted by red (up-regulated genes) and green (down-regulated genes) bars on LGs. DEGs are defined by more than 2-fold change in FPKM values.

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Fig 6. Distribution of CpBV-H4 ChIPs around the 15 joining sites of CpBV-H4 on *P. xylostella* genome. In each joining site, six neighboring genes around a main target site denoted by red-colored gene were selected. Absolute numbers of ChIP reads were counted on six gene bodies and their intergenic regions. Changes in gene expression levels (FPKM values) of these seven genes after CpBV-H4 expression were depicted in lower graph of each panel, in which negative sign indicates decreased gene expression. All gene names and acronym are described in S4 Table.

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Fig 7. Positional effect of the 15 CpBV-H4 joining sites on regulation of host gene expression. ChIPs against CpBV-H4 were counted on gene body ('GB'), upstream ('UP'), and downstream ('DOWN'). (A) Frequency of CpBV-H4 ChIPs around 75 target genes. (B) Number of host genes differentially regulated in expression level after CpBV-H4 expression in their three different genic regions. Differentially expressed genes are defined by at least 2-fold change (FC) in FPKM levels. Gene names and acronym are listed in S4 Table.

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determined using forward primer 5′–GGATCCATGGGAAGAGGATTGGCAA–3′ and reverse primer 5′–GAATTCTCAACCTCCATAACCATA GATC–3′. Total RNA of larvae expressing vH4 or vH4T was extracted using Trizol reagent described below for subsequent RNA-Seq analysis.

**RNA extraction for RNA-Seq analysis**

Total RNA was isolated from four groups of *P. xylostella* larvae (P7, NP5, vH4-expressing larvae, and vH4T-expressing larvae) using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Extracted RNA was resuspended in 40 μL of diethyl pyrocarbonate-treated water. RNA integrity for subsequent RNA-Seq was analyzed using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The RNA QC was evaluated based on RNA Integrity Number (RIN) value greater than or equal to 7. Our samples had RIN values of 7.7 to 8.5.

Using these total RNAs, cDNA library was constructed with Truseq RNA kit (Invitrogen, Seoul, Korea) and sequenced on Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, USA) with 101 bp pair-end sequencing. Raw reads were trimmed using Trimmomatic 0.32 program (http://www.usadellab.org/cms/?page=trimmomatic) under a criterion of 230 (phred score base quality 30% or more). They were then mapped onto DBM genome (http://iae.fafu.edu.cn/DBM/index.php) using TopHat program (version 2.0.13) (http://ccb.jhu.edu/software/tophat/index.shtml) and Bowtie (version 2.2.3). From these mappings, NP5, P7, transient expression of CpBV-H4, and transient expression of truncated CpBV-H4 samples had 55.2%, 26%, 51.9%, and 52.1% mapping ratios, respectively (S1 Table). These mapped reads were then assembled with Cufflinks (version 2.2.1) (http://cole-trapnell-lab.github.io/cufflinks/). The assembled contigs were used to calculate FPKM (fragment per kilobase of transcript per million mapped reads). They were then annotated with DBM database.

**Differentially expressed gene (DEG) transcript analysis**

For DEG analysis of four treatment groups described in section 2.3, a total of 12,945 transcripts were used by deleting 5,128 transcripts from a total of 18,073 transcripts because at least one of these samples contained FPKM values of 0. DEG used a criterion of at least two-fold change in FPKM value.

**Chromatin immunoprecipitation and deep sequencing (ChIP-Seq)**

ChIP-Seq was performed using the above-mentioned four treatment groups of *P. xylostella*. A polyclonal antibody [17] for CpBV-H4 was used for ChIP. Briefly, 50 larvae for each group were homogenized in 1% sodium dodecyl sulphate followed by addition of formaldehyde (final concentration of 1%) to fix DNA with protein and then sonicated for 30 cycles for 1 min with 2 min interval. After sonication, DNA fragments in the range of 200–300 bp in 200 μL were used for ChIP-Seq according to QuickChIP kit manual (Cat. No. NBP2-29902, Novus Biologicals, Littleton, CO, USA). Briefly, 200 μL of sheared DNA was added to 800 μL of ChIP dilution buffer containing 75 μL of salmon sperm DNA (Sigma, St. Louis, MO, USA). CpBV-H4 antibody (1 μg) was added along with Protein A/G agarose (Thermo Scientific, Waltham, MA, USA). The complex of captured chromatin was then dissociated into protein and DNA using Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The precipitated DNA was then resuspended in the same Tris-EDTA buffer and subjected to sequencing using Illumina HiSeq2000 platform with paired-end sequencing mode.
Screening of major incorporation sites of CpBV-H4 on host chromatin from ChIP-Seq

Four ChIP-Seq data were used to determine CpBV-H4 joining sites. All ChIP sites were validated by significance value to discriminate specific and nonspecific bindings. To compare P7 and NP5 ChIP-Seq sites, significance at E value of $10^{-8}$ was used. Remaining validated ChIP sites were annotated by their nearest genes. Parasitism-specific ChIP sites were determined by manually deleting overlapping genes between two ChIP sites derived from P7 and NP5 larvae. To compare vH4 and vH4T ChIP-Seq sites, significance at E value of $10^{-13}$ was used due to relatively higher mapping ratios compared to that in the comparison between P7 and NP5 ChIPs. Remaining validated ChIP sites were annotated by their nearest genes. CpBV-H4-specific ChIP sites were determined by manually deleting overlapping genes between vH4 and vH4T ChIP sites. Major incorporation sites of CpBV-H4 were then determined by overlapping sites between parasitism-specific ChIP sites and CpBV-H4-specific ChIP sites.

Physical mapping of ChIP targets on \textit{P. xylostella} chromosome

Total chromosomal map (394 Mb) assigned into 31 linkage groups (LGs) was downloaded from DBM database (http://ise.fafu.edu.DBM/chrList.php) divided into 171 scaffolds (111.9 Mb). For physical mapping, locations of all 51 ChIP targets were identified using FGENESH program (http://www.softberry.com). The ORFs of \textit{P. xylostella} were predicted using \textit{Bombyx mori} ORFs as templates. All 51 CpBV-H4 ChIP-Seq targets were physically mapped onto 31 LGs of \textit{P. xylostella}.

In-depth localization analysis of major incorporation sites of CpBV-H4

Among 51 ChIP-Seq targets with respect to CpBV-H4, 15 targets were located on 11 characterized LGs. In-depth analyses of these 15 CpBV-H4 ChIP-Seq targets were done by choosing three ORF nearest to both sides of main ChIP-Seq targets. The total numbers of ChIPs were then counted on gene body (‘GB’, a region containing open reading frame), 5’ region (‘upstream’) from GB, and 3’ region (‘downstream’) from GB, in which downstream and upstream regions were intergenic regions. The fold changes in FPKM for all 105 ORFs were obtained from RNA-Seq DEG data between P7 vs NP5.

Screening of parasitism-specific and CpBV-H4-specific genes

Four treatment group samples were used for the selection of parasitism-specific (P7 vs NP5) and CpBV-H4-specific (vH4 vs vH4T) genes. The total number of genes expressed in both groups was separated based on up-regulation or down-regulation. In each regulation group, genes exhibiting more than two-fold change were chosen. These genes were then compared to the overlapped genes between P7 and NP5 to select parasitism-specific genes or compared to overlapped genes between vH4 and vH4T to select CpBV-H4-specific genes.

ChIP sequence analysis for CpBV-H4 targets against P7 chromatins

Joining sites (133–155 nucleotides) of CpBV-H4 against chromatins of parasitized larvae were further selected based on E-value criterion at $10^{-8}$. All 538 target sequences were then aligned using MegAlign of Lasergene to identify repeat sequences and consensus motifs. AT ratios of targets were estimated using ENDMEMO program (http://www.endmemo.com/bio/gc.php). Consensus sequences were functionally annotated from DBM database (http://iae.fafu.edu.cn/DBM/index.php) using MOTIF program (www.genome.jp/tools/motif/).
Supporting information

S1 Table. RNA-Seq summary of different treatment groups of *P. xylostella* larvae. Treatments includes *in vivo* transient expression of CpBV-H4 (‘vH4’), truncated CpBV-H4 (‘vH4T’), parasitized larvae at day 7 (‘P7’), nonparasitized larvae at day 5 (‘NP5’). (DOCX)

S2 Table. Functional prediction of repeat motifs found in CpBV-H4 joining sites. The prediction used MOTIF program (www.genome.jp/tools/motifs/). (DOCX)

S3 Table. Influence of distribution of *CpBV-H4* joining sites on expression of nearest genes. The *CpBV-H4* location was determined by the most significant ChIP assay. The locations of gene body (‘GB’), UPSTREAM (‘UP’), and downstream (‘DOWN’) were with respect to the nearest coding DNA sequence (CDS). (DOCX)

S4 Table. Full name and their acronyms used in Fig 6. (DOCX)

S1 Fig. Number of ChIP-Seq targets in four different treatments including *in vivo* transient expression of CpBV-H4 (‘vH4’), truncated CpBV-H4 (‘vH4T’), parasitized larvae at day 7 (‘P7’), and nonparasitized larvae at day 5 (‘NP5’). (TIF)

S2 Fig. Repeat sequences in some joining sites of CpBV-H4. (TIF)

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References

1. Webb BA, Beckage NE, Hayakawa Y, Krell PJ, Lanzrein B, Stoltz DB, et al. (2000) Polydnaviridae. In Virus taxonomy (van Regenmortel MHV, Maniloff J, Mayo MA, McGeoch DJ, Preingle CR & Wickner RB, eds.), pp. 253–260. Academic Press, New York.

2. Bezier A, Herbinie J, Lanzrein B, Drezen JM (2009) Polydnavirus hidden face: the genes producing virus particles of parasitic wasps. J Invertebr Pathol 101: 194–203. https://doi.org/10.1016/j.jip.2009.04.006 PMID: 19460382

3. Strand MR, Burke GR (2013) Polydnavirus-wasp associations: evolution, genome organization, and function. Curr Opin Virol 3: 587–594. https://doi.org/10.1016/j.coivo.2013.06.004 PMID: 23816391

4. Burke GR, Strand MR (2014) Systematic analysis of a wasp parasitism arsenal. Mol Ecol 23: 890–901. https://doi.org/10.1111/mec.12648 PMID: 24383716

5. Bae S, Kim Y (2004) Host physiological changes due to parasitism of a braconid wasp, Cotesia plutellae, on diamondback moth, Plutella xylostella. Comp Biochem Physiol A 138: 39–44.

6. Ibrahim AM, Kim Y (2006) Parasitism by Cotesia plutellae alters the hemocyte population and immunological function of the diamondback moth. Plutella xylostella. J Insect Physiol 52: 943–950. https://doi.org/10.1016/j.jinsphys.2006.06.001 PMID: 16872627

7. Kwon B, Song S, Choi JY, Je YH, Kim Y (2010) Transient expression of specific Cotesia plutellae bracoviral segments induces prolonged larval development of the diamondback moth, Plutella xylostella. J Insect Physiol 56: 650–658. https://doi.org/10.1016/j.jinsphys.2010.01.013 PMID: 2138886

8. Kumar S, Kim Y. Revision of Cotesia plutellae bracovirus genes and RNA-Seq: their persistent expression in infected host. Insect Sci (submitted).

9. Burke GR, Strand MR (2015) Polydnaviruses: from discovery to current insights. Virology 10: 393–402.

10. Kim Y, Choi Y, Je YH (2007) Cotesia plutellae bracovirus genome and its function in altering insect physiology. J Asia Pac Entomol 10: 181–191.

11. Gad W, Kim Y (2008) 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. https://doi.org/10.1099/vir.0.83585-0 PMID: 18343834

12. Gad W, Kim Y (2009) N-terminal tail of a viral histone H4 encoded in Cotesia plutellae bracovirus is essential to suppress gene expression of host histone H4. Insect Mol Biol 18: 111–118. https://doi.org/10.1111/j.1365-2583.2009.00860.x PMID: 19196351

13. Hepat R, Kim Y (2011) Transient expression of a viral histone H4 inhibits expression of cellular and humoral immune-associated genes in Tribolium castaneum. Biochem Biophys Res Commun. 2011; 415: 279–283. https://doi.org/10.1016/j.bbrc.2011.04.040 PMID: 22037579

14. Kumar S, Venkata P, Kim Y. Suppressive activity of a viral histone H4 against two host chromatin remodeling factors: lysine demethylase and SWI/SNF. J Gen Virol. 2016; 97: 2780–2796. https://doi.org/10.1099/jgv.0.000560 PMID: 27443989

15. Kim G, Kim Y. Up-regulation of circulating hemocyte population in response to bacterial challenge is mediated by octopamine and 5-hydroxytryptamine via Rac1 signal in Spodoptera exigua. J Insect Physiol. 2010; 56: 559–566. https://doi.org/10.1016/j.jinphys.2009.11.022 PMID: 19961854

16. Hepat R, Kim Y. A viral factor, CpBV15a, interacts with a translation initiation factor, eIF2, to suppress host gene expression at a post-transcriptional level. J Invertebr Pathol. 2013; 114: 34–41. https://doi.org/10.1016/j.jip.2013.05.004 PMID: 23711415

17. Hepat R, Song JJ, Lee D, Kim Y. A viral histone h4 joins to eukaryotic nucleosomes and alters host gene expression. J Virol. 2013; 87: 11223–11230. https://doi.org/10.1128/JVI.01759-13 PMID: 23926351

18. Baxter SW, Davey JW, Johnston JS, Shelton AM, Heckel DG, Jiggins CD, et al. Linkage Mapping and Comparative Genomics Using Next-Generation RAD Sequencing of a Non-Model Organism. PLoS ONE. 2011; 6: e19315. https://doi.org/10.1371/journal.pone.0019315 PMID: 21541297

19. Provost B, Jouan V, Hilliou F, Delobel P, Bernardo P, Ravalle M. et al. Lepidopteran transcriptome analysis following infection by phylogenetically unrelated polydnaviruses highlights differential and common responses. Insect Biochem Mol Biol. 2011; 41: 582–591. https://doi.org/10.1016/j.ibmb.2011.03.018 PMID: 21457783
20. Etebari K, Palfreyman RW, Schlipalius D, Nielsen LK, Glatz RV, Asgari S. Deep sequencing-based transcriptome analysis of *Plutella xylostella* larvae parasitized by *Diadegma semiclausum*. BMC Genomics 12: 446. https://doi.org/10.1186/1471-2164-12-446 PMID: 21906285

21. Song KH, Jung MK, Eum JH, Hwang IC Han SS (2008) Proteomic analysis of parasitized *Plutella xylostella* larvae plasma. J Insect Physiol 54: 1270–1280. https://doi.org/10.1016/j.jinsphys.2008.06.010 PMID: 18671979

22. Bae S, Kim Y (2009) Ikk genes encoded in Cotesia plutellae bracovirus suppress an antiviral response and enhance baculovirus pathogenicity against the diamondback moth, *Plutella xylostella*. J Invertebr Pathol 102: 79–87. https://doi.org/10.1016/j.jip.2009.06.007 PMID: 19559708

23. Surakasi VP, Nalini M, Kim Y (2011) Host translational control of a polydnavirus, Cotesia plutellae bracovirus, by sequestering host eIF4A to prevent formation of a translation initiation complex. Insect Mol Biol 20: 609–618. https://doi.org/10.1111/j.1365-2583.2011.01091.x PMID: 21699595

24. Kim E, Kim Y (2016) Translational control of host gene expression by a cys-motif protein encoded in a bracovirus. PLoS ONE 11: e0161661. https://doi.org/10.1371/journal.pone.0161661 PMID: 27598941

25. Basio NAM, Kim Y (2008) Additive effect of teratocyte and calyx fluid from Cotesia plutellae on immunosuppression of *Plutella xylostella*. Physiol Entomol 31: 341–347.

26. Ali R, Kim Y (2013) Teratocyte-secreting proteins of an endoparasitoid wasp, *Cotesia plutellae*, prevent host metamorphosis by altering endocrine signals. J Invertebr Pathol 166: 251–262.

27. Webb BA, Luckhart S (1996) Evidence for an early immunosuppressive role for related *Campoletis sonorensis* venom and ovarian proteins in *Heliothis virescens*. Arch Insect Biochem Physiol 26: 147–163.

28. Asgari S (2006) Venom proteins from polydnavirus-producing endoparasitoids: their role in host-parasite interactions. Arch Insect Biochem Physiol 61: 146–156. PMID: 16482579

29. Luckhart S, Webb BA (1996) Interaction of a wasp ovarian protein and polydnavirus in host immune suppression. Dev Comp Immunol 20: 1–21. PMID: 8738933

30. Shi M, Zhao S, Wang ZH, Stanley D, Chen XX (2016) *Cotesia vestalis* parasitization suppresses expression of a *Plutella xylostella* thioredoxin. Insect Mol Biol. 25: 679–688. https://doi.org/10.1111/imb.12252 PMID: 27376399

31. Hepat R, Kim Y (2012) *In vivo* transient expression for the functional analysis of polydnaviral genes. J Invertebr Pathol 111: 152–159. https://doi.org/10.1016/j.jip.2012.07.025 PMID: 22984446

32. Kim J, Hepat R, Lee D, Kim Y (2013) Protein tyrosine phosphatase encoded in Cotesia plutellae bracovirus suppresses a larva-to-pupa metamorphosis of the diamondback moth, *Plutella xylostella*. Com Biochem Physiol A 166: 60–69.

33. Black JC, Allen A, Van RC, Forbes E, Longworth M, Tschöp K. et al. (2010) Conserved antagonism between JMJD2A/KDM4A and HP1gamma during cell cycle progression. Mol Cell 40: 736–748. https://doi.org/10.1016/j.molcel.2010.11.008 PMID: 21145482

34. Armstrong JA, Papoulas O, Daubresse G, Sperling AS, John TL, Matthew PS, et al. (2002) *Drosophila* BRM complex facilitates global transcription by RNA polymerase II. EMBO J 19: 5245–5254.

35. Qi Y, Teng Z, Gao L, Wu S, Huang J, Ye G, et al. (2015) Transcriptome analysis of an endoparasitoid wasp *Cotesia chilonis* (*Hymenoptera: Braconidae*) reveals genes involved in successful parasitism. Arch Insect Biochem Physiol 88: 203–221. https://doi.org/10.1002/arch.21214 PMID: 25336406