Characterization of two genes of *Cotesia vestalis* polydnavirus and their expression patterns in the host *Plutella xylostella*

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*Cotesia vestalis* is an endoparasitoid of larval stages of *Plutella xylostella*, the diamondback moth. For successful parasitization, this parasitoid injects a polydnavirus into its host during oviposition. Here we isolated two genes, which we named CvBV1 and CvBV2. CvBV1 was located on segment CvBV-S5 with a size of 790 bp, while CvBV2 was located on segment CvBV-S51 with a size of 459 bp. A gene copy of CvBV2 was found on segment CvBV-S48, which we name CvBV2'. Gene duplication occurred in both genes, tandem gene duplication for CvBV1 and segmental duplication for CvBV2. Gene transcripts of the two genes were detected in hosts as early as 0.5 h post-parasitization (p.p.) and continued to be detected for six days, and tissue-specific expression patterns showed that they could be detected in the haemolymph and brain at 2 h p.p., suggesting that they could participate in early protection of parasitoid eggs from host cellular encapsulation.

Polydnavirus (PDV) is a unique group of insect viruses because of their obligate and symbiotic associations with parasitic wasps in the families *Braconidae* and *Ichneumonidae*, as *Bracovirus* (BV) and *Ichnovirus* (IV) genera, respectively (Kroemer & Webb, 2004). They are so named because their genome is polydisperse, containing a series of different circular DNAs (Turnbull & Webb, 2002). The endoparasitoid, *Cotesia vestalis* (Hymenoptera: Braconidae), employs multiple strategies of active and passive immune-suppression in overcoming the defences of its host, *Plutella xylostella* (Lepidoptera: Plutellidae). We have demonstrated previously that the venom from *C. vestalis* has a limited effect on haemocytes, whereas calyx fluid from *C. vestalis* may play a major role in the suppression of the host immune system and the venom probably synergizes the effect of calyx fluid or *C. vestalis* polydnavirus (CvBV) (Yu *et al.*, 2007). Most of the CvBV genome has been sequenced using the plasmid capture system (PCS), although the segments over 30 kb do not amplify due to PCS system restrictions (Choi *et al.*, 2005). CvBV shares some genetic similarity with the *Cotesia congregata* bracovirus (CcBV), the first BV genome to be fully sequenced (Espagne *et al.*, 2004).

Here we isolated two CvBV genes and their genomic organization and expression patterns were investigated.

The GenBank accession numbers of the sequences reported in this paper are EF467277 and EF467278. Sequences of the primers used in this study are available with the online version of this paper.
predicted to encode proteins using Fgenesh (Salamov & Solovyev, 2000; Solovyev & Salamov, 1999) and were named CvBV1-s5 and CvBV1-s11, respectively (Fig. 1a).

Gene duplication has been previously reported in BVs and IVs and categorized as follows: (1) tandem gene duplication, (2) segmental duplication or (3) unresolved between the two hypotheses (Friedman & Hughes, 2006; Hilgarth & Webb, 2002; Provost et al., 2004). The pair of CvBV1 and CvBV1-s5 favoured the hypothesis of tandem gene duplication since both genes clustered on the same segment, whereas CvBV2/CvBV2’ and CvBV-HP5107/CvBV-HP4805 depicted segmental duplication. Gene duplication along with genome segmentation, segment nesting and non-equimolar segment ratios are different strategies adopted by the virus to increase the copy number of essential genes and the levels of gene expression in the absence of virus replication (Kroemer & Webb, 2004; Webb & Cui, 1998). The current results revealed a high degree of similarity between segments CvBV-S51 and CvBV-S48, and between segments CvBV-S5 and CvBV-S11. Despite the close resemblances, Choi et al. (2005) made it clear that segments CvBV-S51 and CvBV-S48, CvBV-S5 and CvBV-S11 are all different segments with different digestion patterns.

CvBV1 had a size of 790 bp and was composed of two exons and an intron, which was consistent with the prediction in GenBank. In the 3’ UTR, two potential polyadenylation signals (Beaudoing et al., 2000) were observed (Fig. 1b). CvBV2 had a size of 459 bp and was similarly composed of two exons and an intron, but the first exon was not consistent with the prediction in GenBank. There were three potential polyadenylation signals in the 3’ UTR (Fig. 1c). The complicated genome organization and gene expression patterns make PDV gene prediction difficult, so different parameters may be adopted for PDV genes expressed in different hosts (hymenopteran and lepidopteran). In order to improve PDV gene prediction, more comprehensive PDV gene transcription, processing and expression models are needed (Gundersen-Rindal & Pedroni, 2006). In the current study gene prediction was carried out using Fgenesh (Solovyev & Salamov, 1999; Salamov & Solovyev, 2000) with human, Drosophila melanogaster, honey bee and Brugia malayi (parasitic nematode) settings. Most settings correctly predicted the nature of CvBV1, except for the Drosophila...
settings, whereas CvBV2 could only be predicted correctly using the Brugia malayi settings. Brugia malayi, a mosquito-borne nematode parasite and a cause of lymphatic filariasis in humans (Ottesen et al., 1997), has evolved specific measures to counter host immune defences—strategies quite similar to those employed by PDVs, which is a possible reason for the correct prediction by this species.

The open reading frame (ORF) of CvBV1 comprises 97 aa encoding a protein with a predicted molecular mass of 10.9 kDa (Fig. 1b). Computer analyses of the deduced amino acid sequence suggested that the protein could have a signal peptide (Nielsen et al., 1997), indicating that CvBV1 protein was probably secreted from CvBV-infected cells. The most likely cleavage sites of putative signal peptides were between positions 24 and 25 (SGS-SP) or between positions 31 and 32 (AYA-KP). A potential O-glycosylation site at position 94 in the deduced protein was predicted by NetOGlyc 3.1 (http://www.cbs.dtu.dk/services/NetOGlyc/). The search for conserved domains using Blocks (Henikoff & Henikoff, 1994) indicated significant similarity to a staphylococcal enterotoxin, which has been shown to induce apoptosis in thymocytes, which are a kind of immune cell (Lin et al., 1999). The ORF of CvBV2 encoded 91 amino acids with a predicted molecular mass of 10.0 kDa (Fig. 1c). The predicted protein also seemed to have a N-terminal signal peptide and was proposed to be localized in the lysosome (Nakai & Horton, 1999). The predicted cleavage sites of the putative signal peptides were between positions 18 and 19 (GTS-WF) or between positions 30 and 31 (VDA-LP) (Nielsen et al., 1997). There were three potential N-glycosylation sites predicted by NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/).

**Fig. 2.** Sequence comparison of CvBV1 and CvBV2 with homologous hypothetical proteins found in CcBV, CvBV and GiBV. GenBank accession numbers: YP_184861.1 for CcBV_23.3, ABK63302.1 for CvBV-HP1002, AAZ04271.1 for CvBV-HP401, ABK63336.1 for CvBV-HP3701, YP_184904.1 for CcBV_33.6, YP_184802.1 for CcBV_9.3, ABK57021.1 for GIP_L1_00350, ABK57038.1 for GIP_L1_00520, AAZ04270.1 for CvBV-HP302, YP_184803.1 for CcBV_9.4, YP_184902.1 for CcBV_33.4, ABK63358.1 for CvBV-HP4805, ABK63370.1 for CvBV-HP5107, ABK57050.1 for GIP_L1_00640, and ABK57047.1 for GIP_L1_00610.
and show a high degree of similarity (68 %), indicating that CcBV_23.3 might be an equivalent gene in CcBV. CcBV2 shares 57 % similarity with CcBV-HP5107 and CcBV-HP4805 over 84 aa. The CLUSTAL_X alignment shown in Fig. 2 revealed that there were two conserved domains on sequences of homologues for both genes, with one domain located on the N-terminal region and the other located on a non-secretive region. The peptide sequence functions like a postal address on an envelope by targeting proteins for secretion or for transfer to specific organelles for further processing (Choo et al., 2005); therefore we suggest that these homologues are transferred to the same location and share similar functions. Several other hypothetical proteins from CcBV, CcBV, GiBV or Cotesia kariyai polydnavirus (CKBV) only contained the conserved N-terminal domains, indicating that they might be transferred to the same destination but had different functions. For phylogenetic analysis, neighbour-joining trees were generated using PAUP4 (Fig. 3a). The result revealed that CvBV1 is closely related to CcBV_23.3, CcBV-HP302, GIP_L1_00350 and GIP_L1_00520, and CcBV2 is closely related to CcBV-HP4805 and CcBV-HP5107 (Fig. 3a). These homologous genes were among CcBV, CcBV and GiBV, suggesting that these BVs are closely related. Transmission of PDVs is exclusively vertical and no replication occurs while inside the host (Stoltz, 1990; Stoltz et al., 1986), so genetic changes occur solely in the female wasp. Therefore, evolution of the polydnavirus is expected to parallel that of the wasp (Friedman & Hughes, 2006). Studies of BV phylogeny indicate that all BV-containing wasps share a common ancestor (Whitfield, 1997). The available fossil-dating techniques reveal that the ancestor of this lineage lived approximately 73.7 ± 10 million years ago (Whitfield, 2002). Phylogenetic relationships among homologues of CvBV1 and CvBV2 reconfirm that the closer the relationship among the wasps, the more genetically similar are their polydnaviruses.

As an advanced step, examination of temporal and tissue-specific expression patterns of CvBV1 and CvBV2 were carried out using non-quantitative RT-PCR with gene-specific RT-PCR primers (Fig. 3b; Supplementary Table S1, available with the online version of this paper). Total RNAs from parasitized P. xylostella larvae at various time points and different tissues were treated with RNase-free DNase (TaKaRa) to eliminate DNA contamination and were used as templates in non-quantitative RT-PCR, which was performed according to the manufacturer’s protocol using an RT-PCR kit (QIAGEN). The absence of contaminating wasps share a common ancestor (Whitfield, 1997). The available fossil-dating techniques reveal that the ancestor of this lineage lived approximately 73.7 ± 10 million years ago (Whitfield, 2002). Phylogenetic relationships among homologues of CvBV1 and CvBV2 reconfirm that the closer the relationship among the wasps, the more genetically similar are their polydnaviruses.

The search for conserved domains using Blocks (Henikoff & Henikoff, 1994) indicated significant similarity to a hydrolase of the glycosyl hydrolase 92 family. Many studies have indicated that lysosomes and lysosomal enzymes, including some hydrolases, are involved in apoptosis (Kroemer & Jaattela, 2005; Tardy et al., 2006). Apoptosis is ubiquitously observed in parasitized hosts. A number of characterized genes of PDV appear to induce apoptosis of haemocytes (Lapointe et al., 2005; Le et al., 2003; Strand & Pech, 1995; Strand et al., 1997), which are involved in cellular immunity of insects and take part in phagocytosis, encapsulation and nodule formation (Lavine & Strand, 2002).

Homologues of CvBV1 and CvBV2 were found among CvBV, CcBV and Gyptapanteles indiensis polydnavirus (GiBV) by BLASTP searches with an E-value cut-off of 10⁻⁸ (Fig. 2). CvBV1 and CcBV_23.3 are the same size (97 aa)
comprise over 10% of the total haemolymph proteins by 24 h p.p. (Beckage et al., 1987; Harwood et al., 1994). Tissue-specific expression patterns of the two genes showed that they could be detected in haemolymph and brain at 2 h p.p. and later they could be detected in the midgut. These findings suggest that these two genes could be involved in early protection of parasitoid eggs from host cellular encapsulation. For CvBV1 and CvBV2, further research will focus on obtaining purified native proteins and determining their mode of action.

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References


