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A copy of cystatin from the diamondback moth *Plutella xylostella* is encoded in the polydnavirus *Cotesia plutellae* bracovirus

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ABSTRACT

Cystatins (CSTs) are reversible and competitive inhibitors of cysteine proteases. Some polydnaviruses encode viral CSTs that have been speculated to play a crucial role in viral pathology. Four CSTs have been reported in the episomal genome of a polydnavirus, *Cotesia plutellae* (synonymous with *C. vestalis*) bracovirus (CpBV). These 4 CSTs share high sequence homologies with other bracoviral CSTs. Further sequence analysis showed that 2 of the CpBV-CSTs are identical. The remaining 3 CSTs have been designated CpBV-CST1, CpBV-CST2, and CpBV-CST3. Expression analysis indicated that CpBV-CST2 was not expressed in any stage of *Plutella xylostella*, either parasitized or non-parasitized by *C. plutellae*. However, both CpBV-CST1 and CpBV-CST3 were expressed in all stages of *P. xylostella*. Interestingly, these 2 genes were also expressed in non-parasitized *P. xylostella* in all developmental stages. A CST sequence from the non-parasitized larva was 100% identical with that of CpBV-CST1 for the entire open reading frame (ORF). To understand the role of CpBV-CST1 in viral pathology, the ORF was cloned into a eukaryotic expression vector and transiently expressed in non-parasitized larvae. The *in vivo* transient expression lasted for at least 4 days. Under this condition, the treated larvae suffered significant suppression in immune responses and in development. These results suggest that CpBV-CSTs play a crucial role in parasitism, altering host immune and developmental processes by interrupting normal interactions between CSTs and cysteine proteases in *P. xylostella*.

Introduction

Polydnaviruses (PDVs) are insect DNA viruses that have a specific symbiotic relationship with some types of endoparasitoid wasps (Beckage and Drezen, 2012). The entire viral genome is located in the host wasp chromosome(s) and is vertically transmitted to subsequent generations of host wasps (Fleming and Summers, 1991). To foster this vertical transmission, PDVs replicate in the host wasp and form episomal viral particles that contain a partial genome, which assists in host wasp development in parasitized lepidopteran hosts (Webb and Strand, 2005). The horizontal transfer of the episomal PDV particles to the lepidopteran hosts occurs during parasitization; however, PDVs do not replicate in the parasitized hosts.

PDVs are divided into 2 genera of bracovirus (BV) and ichnovirus (IV), depending on their wasp host families and viral morphologies (Webb et al., 2000). These 2 genera also show differences in genome composition, suggesting that they have independent origins (Bézier et al., 2007). Analysis of viral coat proteins embedded in wasp chromosomes suggests that BV originated from an ancestral nudivirus that was in a subgroup of baculovirus (Bézier et al., 2009). In contrast, the origin of IV remains unknown (Volkoff et al., 2010). However, in a form of convergent evolution, both BV and IV exhibit similar pathological symptoms by suppressing immune responses and delaying larval development of the parasitized host to assist host wasp development (Strand, 2010). Aside from viral ankyrins, though, BV and IV use different gene family products to alter host physiological processes (Kroemer and Webb, 2004).

An endoparasitoid wasp, *Cotesia plutellae* (synonymous with *C. vestalis*), parasitizes young larvae of *Plutella xylostella* and alters their physiological processes of immune response and development (Bae and Kim, 2004). The wasp possesses a symbiotic PDV called *C. plutellae* BV (CpBV), which alone induces the physiological alterations (Kim et al., 2007). A total of 157 genes are annotated in the episomal viral genome (Chen et al., 2011). Several typical PDV gene families have been detected in CpBV and have been shown to be functional by using a novel technique called segment expression and RNA interference (SERI) (Barandoc et al., 2009). However, the physiological functions of more than half of the annotated genes remain unknown.

Cystatins (CSTs) are reversible and competitive inhibitors of C1A cysteine proteases, corresponding to papain-like cathepsins in plants and animals (Turk et al., 1997; Abrahamson et al., 2003). Cathepsins are synthesized as inactive proenzymes and are activated by proteolytic cleavage at an N-terminal pro-peptide region (Lecaille et al., 2002). In insects, 4 cathepsin types (B, L, F-like, and 26/29 kDa proteins) have been reported (Kurata et al., 1992; Attardo et al., 2006).

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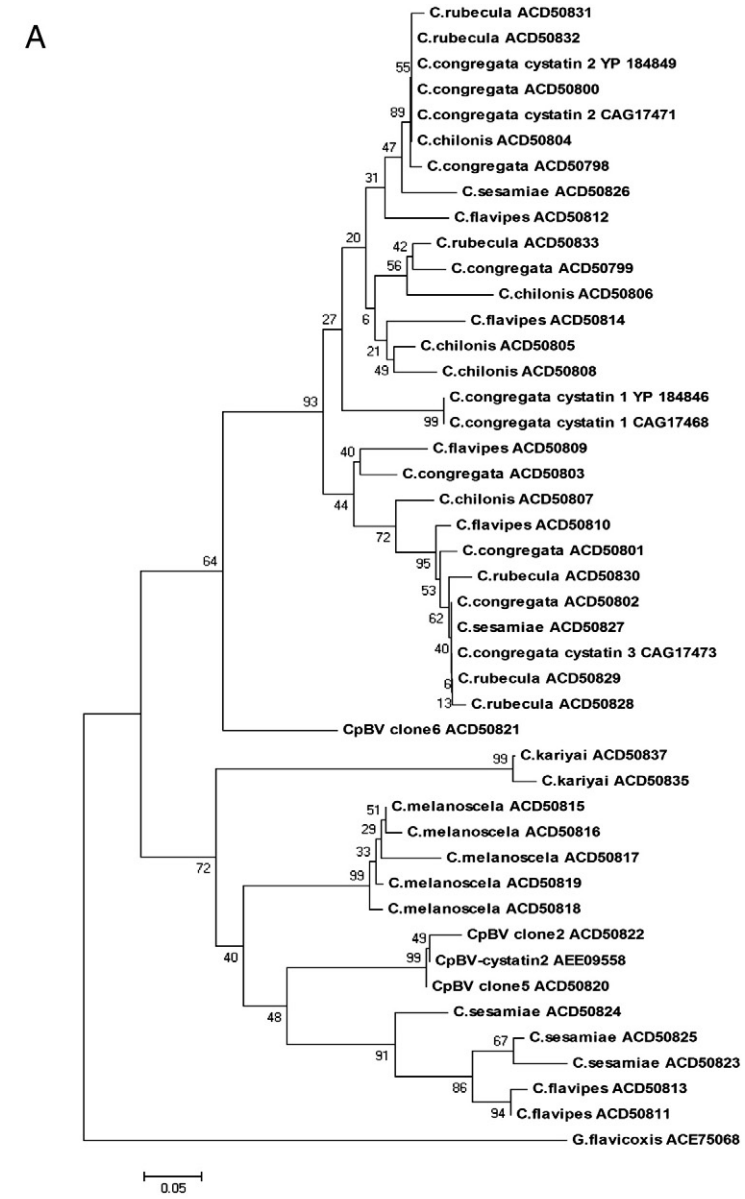
A
C.rubecula ACD50831
C.rubecula ACD50832
C.congregata cystatin 2 YP 184849
C.congregata ACD50800
C.congregata cystatin 2 CAG17471
C.chilonis ACD50804
C.congregata ACD50798
C.sesamiae ACD50826
C.flavipes ACD50812
C.rubecula ACD50833
C.congregata ACD50799
C.chilonis ACD50806
C.flavipes ACD50814
C.chilonis ACD50805
C.chilonis ACD50808
C.congregata cystatin 1 YP 184846
C.congregata cystatin 1 CAG17468
C.flavipes ACD50809
C.congregata ACD50803
C.chilonis ACD50807
C.flavipes ACD50810
C.congregata ACD50801
C.rubecula ACD50830
C.congregata ACD50802
C.sesamiae ACD50827
C.congregata cystatin 3 CAG17473
C.rubecula ACD50829
C.rubecula ACD50828
CpBV clone6 ACD50821
C.kariyai ACD50837
C.kariyai ACD50835
C.melanoscela ACD50815
C.melanoscela ACD50816
C.melanoscela ACD50817
C.melanoscela ACD50819
C.melanoscela ACD50818
CpBV clone2 ACD50822
CpBV-cystatin2 AEE09558
CpBV clone5 ACD50820
C.sesamiae ACD50824
C.sesamiae ACD50825
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G.flavicoxis ACE75068
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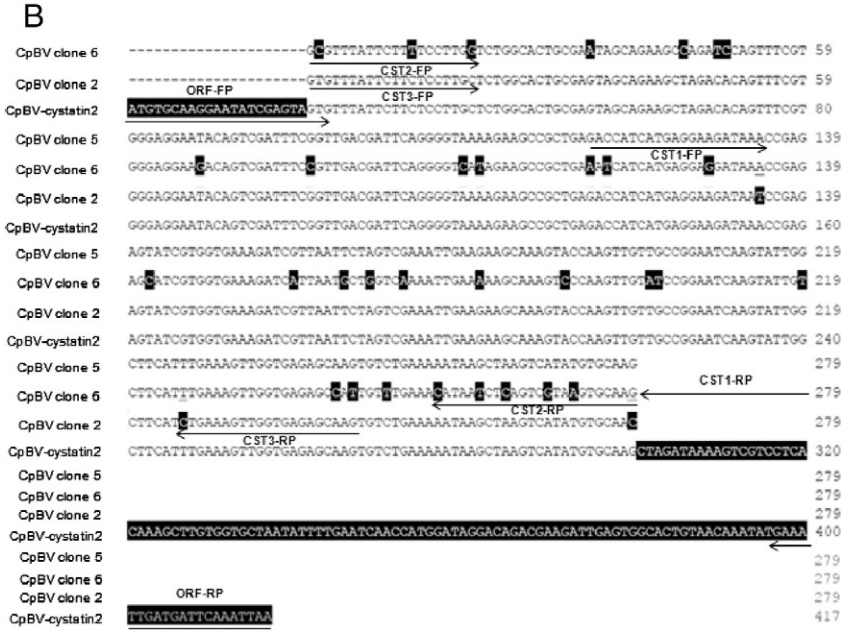
B
CpBV clone 6
CpBV clone 2
CpBV-cystatin2
CpBV clone 5
CST2-FP
ORF-FP
CST3-FP
CST1-FP
CST1-RP
CST2-RP
CST3-RP
ORF-RP