Developmental and Comparative Immunology 44 (2014) 152–162

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Selectivity of a translation-inhibitory factor, CpBV15β, in host mRNAs and subsequent alterations in host development and immunity

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ARTICLE INFO

Article history:
Received 9 November 2013
Revised 3 December 2013
Accepted 3 December 2013
Available online 18 December 2013

Keywords:
Polydnavirus
HTIF
Parasitism
Cotesia plutellae
Plutella xylostella

ABSTRACT

An endoparasitoid wasp, *Cotesia plutellae*, parasitizes young larvae of the diamondback moth, *Plutella xylostella*. Its symbiotic virus, *C. plutellae* bracovirus (CpBV), has been shown to play a crucial role in inducing physiological changes in the parasitized host. A viral gene, *CpBV15β*, exhibits a specific translational control against host mRNAs by sequestering a eukaryotic translation initiation factor, eIF4A. Inhibitory target mRNAs have high thermal stability (>≈9 kcal/mol) of their secondary structures in 5′UTR. To determine the specificity of translational control in terms of 5′UTR complexity, this study screened target/nontarget mRNAs of CpBV15β using a proteomics approach through an *in vivo* transient expression technique. A proteomics analysis of host plasma proteins showed that 12.9% (23/178) spots disappeared along with the expression of CpBV15β. A total of ten spots were chosen, in which five spots ('target') were disappeared by expression of CpBV15β and the other five ('nontarget') were insensitive to expression of CpBV15β, and further analyzed by a tandem mass spectroscopy. The predicted genes of target spots had much greater complexity (−12.3 to −25.2 kcal/mol) of their 5′UTR in terms of thermal stability compared to those (−3.70 to −9.00 kcal/mol) of nontarget spots. 5′UTRs of one target gene (arginine kinase:Px-AK) and one nontarget gene (imaginal disc growth factor:Px-IDGF) were cloned and used for *in vitro* translation (IVT) assay using rabbit reticulocyte lysate. IVT assay clearly showed that mRNA of Px-IDGF was translated in the presence of CpBV15β, but mRNA of Px-AK was not. Physiological significance of these two genes was compared in immune and development processes of *P. xylostella* by specific RNA interference (RNAi). Under these RNAi conditions, suppression of Px-AK exhibited much more significant adverse effects on larval immunity and larva-to-pupa metamorphosis compared to the effect of suppression of Px-IDGF. These results support the hypothesis that 5′UTR complexity is a molecular motif to discriminate host mRNAs by CpBV15β for its host translational control and suggest that this discrimination would be required for altering host physiology to accomplish a successful parasitism of the wasp host, *C. plutellae*.

1. Introduction

Polydnaviruses (PDVs) are a group of unique double-stranded DNA viruses symbiotic to some endoparasitoid wasp (Webb et al., 2000). It comprises of two genera, Ichnovirus (IV) and Bracovirus (BV), depending upon their host wasp family and virion structure (Strand, 2010). Under independent origins of these two viral families, BV is known to be originated from an ancestral nudiviruses, while IV is yet unknown (Bézier et al., 2009; Volkoff et al., 2010).

PDVs are present in a proviral form on host wasp chromosome(s) (Fleming and Summers, 1986; Gruber et al., 1996; Savary et al., 1999). Thus their vertical transmission follows successive generations of host wasps. They also exhibit horizontal transmission from wasp host to lepidopteran host by forming viral particles through replication (Espagne et al., 2004). The viral replication occurs at a late pupal stage of female wasps in their ovarian calyx area (Norton and Vinson, 1983; Volkoff et al., 1995). However, the viral particles contain a partial viral genome, which lacks viral replication-related machinery, and so do not replicate any more in lepidopteran host (Krell and Stoltz, 1980; Krell, 1991). The PDV viral particles then move to the hemocoel of lepidopteran host along with parasitoid wasp eggs during parasitization and enter target tissues (Strand and Pech, 1995; Burke and Strand, 2012). In target tissues, the viral genes are expressed and alter host physiological processes, such as immune responses and immature development (Beckage, 1998; Webb and Strand, 2005). Especially, most PDV genes are known to suppress cellular immune responses to prevent parasitoid eggs from encapsulation of lepidopteran host hemocytes (Turnbull and Webb, 2002; Beckage and Gelman, 2004; Kim et al., 2013). They also extend larval period by suppressing juvenile hormone degradation or ecdysteroid hormone synthesis (Soller and Lanzrein, 1996; Cusson et al., 2001; Pennacchio et al., 2001).

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0145-305X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dci.2013.12.001

Cotesia plutellae (Braconidae: Hymenoptera) is a solitary endoparasitoid wasp that parasitizes young larvae of the diamondback moth, *Plutella xylostella* (Bae and Kim, 2004). The parasitized larvae undergo an immunosuppression (Ibrahim and Kim, 2006) and exhibit a prolonged larval development (Lee and Kim, 2008). This wasp possesses a symbiotic PDV called *C. plutellae* bracovirus (CpBV), which plays a crucial role in the parasitism (Kim, 2006). CpBV is predicted to encode 157 genes, which are grouped into different PDV gene families classified into different functional roles in the parasitism (Kim et al., 2007; Chen et al., 2011). For example, *CpBV-PTP* gene family members impair cellular immune responses, such as phagocytosis and encapsulation, probably by altering phosphorylation level of target proteins in hemocytes (Ibrahim et al., 2007; Ibrahim and Kim, 2008). A recent study showed that several CpBV-PTPs are involved in inhibition of larva-to-pupa metamorphosis of *P. xylostella* (Kim et al., 2013). *CpBV-IkBs*, which share sequence homology with *cactus* gene of *Drosophila melanogaster*, also show significant expression during entire parasitization period and significantly suppress antiviral activity of the host (Kim et al., 2007; Bae and Kim, 2009). CpBV also encodes *EP1-like* genes, which inhibit cellular immune responses in *P. xylostella* (Kwon and Kim, 2008). In addition, a viral histone H4, viral lectin, and a cysteine-rich protein also contribute to induce host immunosuppression and developmental alteration (Ibrahim et al., 2005; Nalini et al., 2008; Barandoc and Kim, 2009; Kim et al., 2013).

Some PDVs can inhibit expression of host mRNAs at a post-transcriptional level and have been supposed to encode host translation inhibitory factors (HTIFs) (Shelby and Webb, 1997). Later, 2 cys-motif genes (*VHv1.4* and *VHv1.1*) were identified to act as HTIF to inhibit gene expression at a post-transcriptional level in *Heliothis virescens* parasitized by *Campoletis sonorensis* (Kim, 2005). In CpBV, two homologous genes, *CpBV15α* and *CpBV15β*, have been proposed to exhibit HTIF effects to suppress immune responses of *P. xylostella* (Madanagopal and Kim, 2007). These two genes share sequence homologies with eukaryotic translation initiation factors (eIFs) and their gene products inhibit translation of specific host mRNAs (Nalini and Kim, 2009; Barandoc and Kim, 2010). These CpBV-HTIFs are expressed only in late parasitization period and specifically suppress translation of mRNAs encoding storage proteins by recognition of complexity of secondary structure of 5′UTR through sequestering host eIF4A (Surakasi et al., 2011). However, the specificity of CpBV-HTIF in terms of target mRNAs was not thoroughly investigated and needed to be analyzed against entire host mRNAs.

This study was aimed to investigate the specific HTIF effect of CpBV15β on entire plasma proteins of *P. xylostella*. To do this, CpBV15β was transiently expressed in nonparasitized larvae of *P. xylostella* and the resulting change of protein patterns was analyzed using a proteomics approach. To further analyze their specific inhibitory activity on translation, mRNAs of target and nontarget genes were prepared with or without 5′UTR structure and were expressed under a rabbit reticulocyte lysate *in vitro* translation system. This study also analyzed the physiological significance of a target gene inhibited by CpBV15β compared to nontarget gene in terms of their contribution to parasitism in immunosuppression and developmental alteration. This study provided an evidence for the physiological importance of the inhibitory specificity of CpBV15β in the parasitism.

2. Materials and methods

2.1. Insect rearing and parasitization

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

2.2. cDNA construction and RT-PCR

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and digested with DNase (Promega, Madison, WI, USA). After inactivating DNase at 65 °C for 15 min, the RNAs (1 μg) were reverse-transcribed using RT-PCR PreMix kit (Intron Biotechnology, Seoul, Korea) according to the manufacturer's instruction and subsequently treated with RNase H (Promega). The synthesized cDNA was used as a template for PCR amplification. Control PCR reaction was performed with RNA extract as a template to confirm the absence of DNA contamination. PCRs were conducted with 35 cycles under conditions: 94 °C for 1 min followed by 35 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min and final extension at 72 °C for 10 min.

2.3. Construction of a viral recombinant for transient expression

A full open reading frame (ORF) of CpBV15β was obtained from parasitized *P. xylostella* using two gene-specific primers ('CpBV15βFP' and 'CpBV15βRP', Table 1). After confirming direction, PCR products were cloned into a eukaryotic expression vector, pIB/V5-His TOPO® (Invitrogen), according to manufacturer's instruction.

2.4. Transient expression of CpBV15β in nonparasitized larvae of P. xylostella

Each recombinant pIB vector was mixed with Metafectene PRO transfection reagent (Biontex, Planegg, Germany) according to manufacturer's instruction. Briefly, 3 μl (1.5 μg) of recombinant was mixed with 3 μl of Metafectene reagent and incubated for 20 min at room temperature to allow DNA–lipid complexes to be formed before injection into hemocoel of the third instar larvae of *P. xylostella*. Glass capillary injection needles were made using a micropipette puller PN30 (Narishige, Tokyo, Japan). A total of 500 nl was injected into larval hemocoel at a rate of 100 nl/s using a micro-syringe pump controller (World Precision Instruments, Sarasota, FL, USA) under a microscope (Olympus S730, Tokyo, Japan). Larvae injected with empty vector serves as control.

2.5. Western blotting analysis of transient expression

Plasma protein samples of transiently expressed or control larvae were diluted in 100 mM phosphate buffer saline (PBS, pH 7.4) in a concentration of 2 μg/μl and mixed with the same volume of SDS-denaturing buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol in 62.5 mM Tris–HCl, pH 6.8). After boiling for 5 min at 95 °C, samples (15 μg per lane) were run on 15% SDS–PAGE. For Western blotting, the gels were electro-transferred onto Immunblot™ polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA) (Towbin et al., 1979). After electro-transfer, the membrane was blocked with 5% skim milk for 1 h at room temperature. Then it was washed three times with PBS and incubated with CpBV15β antibody (1:1000 dilution) for 1 h at room temperature. This membrane was washed three times in PBS and incubated with an anti-rabbit-IgG conjugated with alkaline phosphatase (1:2000 dilution, Sigma–Aldrich Korea, Seoul, Korea) for 1 h at room temperature. Again, the membrane was washed three times in PBS and incubated with nitroblue tetrazoleum chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT-BCIP, Sigma–Aldrich Korea).