Bacterial expression and cellular localization of Helicoverpa armigera nucleopolyhedrovirus Orf33 in infected host cells

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Abstract: Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HearSNPV) is a selective, highly infectious pathogen to H. armigera and has been extensively used for the control of this pest. This study presents the bacterial expression and localization of ha33 in infected host cells. The ha33 protein expressed in Escherichia coli had protein size of 17kDa. Western blot analysis using polyclonal antibody showed that the product of ha33 in infected Helicoverpa zea cells (HzAM1) was a 31kDa protein, larger than the theoretically predicted size of 28.4kDa. The confocal laser scanning microscope observation demonstrated that ha33 gene product was localized in the cytoplasm of infected HzAM1 cells beginning at 6 h p.i. and remained throughout the infection.

Keywords: Helicoverpa armigera nucleopolyhedrovirus; ORF33; Expression; Localization
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1 Introduction

The cotton bollworm, H. armigera, is a worldwide pest causing serious damage to a variety of agricultural crops such as cotton, tobacco, pepper, tomato, maize, sorghum and soybean. H. armigera single nucleocapsid nucleopolyhedrovirus (HearSNPV) is a selective, highly infectious pathogen to this insect and has been extensively used for the control of this pest since the first isolation of this pathogen in Hubei province of China[1]. Two isolates of HearSNPV, C1 and G4, were cloned in China[2]. Several genes of HearSNPV have been characterized, such as polyhedrin[3], late gene expression factor 2 (lef-2)[4], basic DNA-binding protein (BDBP)[5], Ha122[6], Ha94[7], P74[8], Ha-VP39[9] and Ha29[10]. However, the functions of many other genes of HearSNPV remain unknown.

HearSNPV ORF33 (ha33) is located between 27566 and 28282bp in the HearSNPV-C1 genome, encoding a putative 238aa protein with predicted molecular weight of 28.4kDa and is transcribed in the opposite orientation of the polyhedrin gene. The ha33 homologues were identified in 21 completely sequenced lepidopteran NPVs and GVs, but so far their functions have not been characterized. In this study, the ha33 was expressed in E. coli and was examined for its localization in HearSNPV infected HzAM1 cells. The results indicated that ha33 protein was localized in the cytoplasm.

2 Materials and Methods

2.1 Virus, insect and cell line

HearSNPV C1 was used for infections and propagated in the Helicoverpa zea cell line, HzAM1[11]. HzAM1 cells were grown in TNM-FH Insect Medium (Sigma-ALDRICH, USA) supplemented with 10% fetal bovine serum (Gibco, BRL). A culture of H. armigera larvae was maintained according to Sun et al[12] for HearSNPV polyhedra production. Routine methods for baculovirus manipulations were followed as described by O'Reilly et al[13].

2.2 Cloning, expression and purification of ha33 protein

The complete HearSNPV ORF33 (717 nts) was amplified by PCR using an upstream primer (5′-AGGATCCATGCGGT GCTCGGGTCTT-3′) incorporating a BamHI site (underlined) and a downstream primer (5′-ACTCGAGTTAGTATTGCCG CTGCAT-3′) with an XhoI site (underlined). The amplified fragment was inserted into pGEM-T easy vector (Promega, USA), followed by digestion with BamHI and XhoI, and was subcloned into the expression vector pGEX-4T-2 (Pharmacia, USA), in-frame with the C-terminal glutathione S-transferase (GST) tag in the plasmid. The recombinant plasmid, designated as pGEX-4T-2-ha33, was transformed into E. coli BL21 cells and fusion protein expression was induced by incubation in the presence of 2mmol/L IPTG when the optical density measurement at 600nm reached ~0.7. The GST-ha33 fusion protein was confirmed with the mouse monoclonal anti-GST antibody (Sigma, USA). The recombinant ha33 protein was purified using the MicroSpin™ GST purification module (Pharmacia, USA).

2.3 Generation of anti-ha33 antiserum

The purified ha33 protein was used as an immunogen to raise ha33-specific antiserum in male rabbits. Five doses each with 200 μg purified GST-ha33 fusion protein were injected to

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rabbits. For the first injection, complete Freund's adjuvant (Sigma, USA) was added, while for the others, incomplete adjuvant (Sigma, USA) was used. The rabbits were boosted on days 21, 35, 49 and 63. Ten days after the last injection, the rabbit were bled by cardiac puncture to collect the serum after clotting of the blood.

2.4 Western blot analysis

Monolayers of HzAM1 cells were cultured and HearSNPV-infected at a m.o.i. of 5 $TCID_{50}$ units per cell. Cells were harvested at 0, 24, 48 and 72 h p.i, then pelleted at 4000r/min and resuspended in PBS. The samples were lysed in SDS-PAGE loading buffer by boiling for 10min and then were separated by SDS-PAGE and transferred onto an Immobilon-P nitrocellulose membrane (Millipore China, Shanghai) by semi-dry transfer cell (TRANS-BLOT SD, Bio-RAD, USA) according to manufacturer's procedure. The membranes were incubated overnight in 2% skimmed milk powder in TBS at 4℃. The membranes were allowed to react with ha33 antiserum diluted 1∶5000 for 1h at room temperature and treated further as described by Ijkel et al[14]. Immunoreactive proteins were visualized using goat anti-rabbit IgG-HPR (SourthernBiotech, AL, USA) following the manufacturer's protocol.

2.5 Confocal laser scanning microscope

HzAM1 cells were grown in a petri dish and infected with wild-type HearSNPV. At 48 h p.i., cells were washed three time in 1 × PBS, fixed with 4% paraformaldehyde in 1 × PBS followed by permeation with 0.2% Triton X-100 in 1 × PBS for 10 min, and allowed to react with the polyclonal antiserum diluted 1∶2000 and protein G fused with enhanced green fluorescent protein (EGFP), following similar methods as described by Spector et al[15]. After treatment the cells were examined under a Zeiss LSM510 confocal laser-scanning microscope for fluorescence detection.

3 Results

3.1 Expression of ha33 protein in E. coli

Ha33 was obtained by PCR and cloned into the vector pGEX-4t-2, an E. coli expression vector with a glutathione S-transferase (GST) Tag. Recombinant plasmid pGEX-4T-2-ha33 was identified by BamHI/XhoI restriction analysis, which confirmed proper insertion of ha33 in pGEX-4T-2. Then pGEX-4T-2-ha33 was sequenced to make sure that there were no mutations in ha33 insertion. The designated pGEX-4T-2-ha33, was transformed into E. coli BL21 cells and fusion protein was expressed. A specific band was detected by SDS-PAGE and Western blot by using an anti-GST antibody (Plate Ⅱ-A). However, the expressed ha33 protein had a smaller size of molecular weight (17kDa) than the predicted size (28.4kDa). The recombinant ha33 protein was purified (Plate Ⅱ-A) for preparation of ha33-specific antiserum.

3.2 Localization of ha33 protein in infected host cells

To investigate the localization of the ha33 protein in the host cells, Western blot analysis of infected host cells was carried out using the ha33-specific antiserum (Plate Ⅱ-B). A 31kDa protein of ha33 was detected in infected cells, and the detected ha33 protein in infected cells was larger than the predicted molecular weight (28.4kDa). The larger actual size of ha33 suggested that ha33 might have been subjected to eukaryotic post-translational modifications such as phosphorylation. Confocal laser scanning microscope detection showed that the Ha29 protein was localized in cytoplasm beginning at 6 h p.i. (Plate Ⅱ-C).

4 Discussion

In this report, we presented the bacterial expression and cellular localization of the ha33 in host cells, the homologues of which were not reported in any other baculoviruses to date.

The ha33 was expressed in the E. coli but with a smaller size of 17kDa, than the predicted size of 28.4kDa. The reason for the modification of expressed ha33 in E. coli is unclear. A possible explanation might be that the expression processes for prokaryotes and eukaryotes are different for this viral gene that is originally expressed in eukaryotic cells (host cells). However, the actual size of the protein expressed in host cells determined by SDS-PAGE was 31kDa, slightly larger than the predicted size (28.4kDa). This suggests that the ha33 protein is modified post-translationally, and is in agreement with the presence of ten potential phosphorylation sites, which were found in the putative ha33 protein by the prediction of NetPhos 2.0 Server online (http://ca.expasy.org), including four serine phosphorylation sites (aa 59 ~ 67, 127 ~ 135, 148 ~ 156 and 184 ~ 192), three threonine phosphorylation sites (aa 86 ~ 94, 112 ~ 120 and 131 ~ 139), three tyrosine phosphorylation sites (aa 92 ~ 100, 151 ~ 159 and 209 ~ 216).

Protein of ha33 was detected in the cytoplasm of infected host cells but not in the nucleus, beginning from 6 h p.i. and remained throughout the infection, suggesting that the ha33 protein is correlated to the events in cytoplasm, which is consistent with the result that ha33 was identified as a structural protein (BV-e31) for the budded virus[16]. Contrastingly, the localization of other baculorvirus genes that may be involved in viral infection cycle in host insect larvae show a protein transporting pattern that the gene products are first detected in the cytoplasm and then transported into nucleus, such as BV/ODV-E26, BV/ODV-C42 and ODV-E66[17,18]. However, to know the whole functions of ha33 and its homologues, further studies are needed.

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棉铃虫核型多角体病毒ORF33基因的原核表达和在宿主细胞中亚细胞定位

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摘 要 对棉铃虫 Helicoverpa armigera 核型多角体病毒 HearSNPV 的 ORF33 基因(ha33)进行克隆和原核表达,hass 在 E.coli 中表达不完全,表达产物的大小为 17kDa,小于预测的分子量 28.4kDa。用纯化的原核表达产物免疫家兔,制备了多克隆抗体,应用多克隆抗体检测了 HearSNPV 感染的宿主细胞(HzAMl)中 ORF33 基因的表达,表达产物的分子量为 31kDa。并通过共聚焦荧光显微镜方法,用多克隆抗体检测编码的蛋白在宿主细胞(HzAM1)中的亚细胞定位,发现 ha33 编码的蛋白存在于宿主细胞的细胞质中,并持续到感染后期。

关键词: HearSNPV; ORF33; 表达; 亚细胞定位
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