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Characterization of ORF2 and its encoded protein of the *Helicoverpa armigera* nucleopolyhedrovirus

Yingchao Nie [a,b,1], Qian Wang [a,c,1], Changyong Liang [a,c], Minggang Fang [a], Zehua Yu [b], Xinwen Chen [a,*]

[a] *State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China*
[b] *Institution of Entomology, Central China Normal University, Wuhan 430070, PR China*
[c] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*

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Abstract

The open reading frame 2 (*ha2*) of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV), a conserved gene in most baculoviruses from lepidopteran insects such as p78/83 of the *Autographa californica* MNPV, was characterized. It is 1242 bp long and potentially encodes a 45.9 kDa. *Ha2* is conserved among baculoviruses from lepidopteran insects. *Ha2* transcripts were detected from 16 to 96 h post infection (hpi) of HzAM1 cells. Rabbit polyclonal antiserum against a GST-HA2 fusion protein reacted with three protein of 50, 46 and 35 kDa at 24–72 hpi of HzAM1 cells. Anti OpMNPV ORF2 (homologue of HA2) antibody reacted only with the 46 and 35 kDa proteins in HaSNPV-infected cells. These results demonstrate that Ha2 is modified at the mRNA or protein levels. Western blot analysis showed that only the 50 kDa product of HA2 is a structural component of proteins of both the budded virus (BV) and occlusion-derived virus (ODV) phenotypes. HA2-EGFP fusion protein showed that HA2 is localized primarily in the nucleus of HzAM1 infected cells. The HA2 was found to co-localize with actin by labelling of actin with Rhordamine-Phalloidin. In summary, the data indicated that HA2 is a structural protein and interacts with host cell actin.

Keywords: HaSNPV; p78/83; Ha2; WASP; Baculovirus; Structural protein

1. Introduction

The *Baculoviridae* is a large family of diverse arthropod specific insect viruses with large, circular, supercoiled, double-stranded DNA genomes, and occluded, rod-shaped virions. It consists of two genera: *Granulovirus* (GV) and *Nucleopolyhedrovirus* (NPV), the latter of which are sub-divided into Group I and Group II based on phylogenetic analysis (Chen et al., 1999; Herniou et al., 2001). There are two phenotypes of virion generated during the infection of baculoviruses: budded virus (BV) and occluded virus (ODV). ODV initiates the infection through oral ingestion of virus-contaminated food and BV spreads infection within larvae to different cells and tissues.

Release of BV nucleocapsids from endosomes into the cytoplasm induces the formation of thick actin cables (Charlton and Volkman, 1993). These cables are detected only transiently from 1 to 4 h post infection (hpi), during which time the entire viral nucleocapsid travels to and enters the nucleus (Granados, 1980). During this period, viral nucleocapsids are often co-localized with one end of an actin cable while the other end is oriented away from the virion (Charlton and Volkman, 1993). It was shown that nucleocapsids of the *Autographa californica* nucleopolyhedrovirus (AcMNPV) are capable of nucleating actin polymerization in vitro and two viral capsid proteins, P39 and P78/83, were found to bind actin directly (Lanier and Volkman, 1998). The protein P78/83, a nucleocapsid-associated phosphoprotein (Russell et al., 1997), shares the WH2 and acidic motif with mammalian Wiskott-Aldrich syndrome protein (WASP) family, although the overall arrangement of the WH2 and acidic motifs in P78/83 homolog is somehow different from that of their counterparts in WASP-family proteins (Machesky et al., 2001). Therefore, the conservation between P78/83 homologues and

* Corresponding author. Tel.: +86 27 87199106; fax: +86 27 87199106.
E-mail address: chenxw@pentium.whiov.ac.cn (X. Chen).
[1] They contributed equally to this paper.

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WASP-family proteins suggests that baculoviruses might use the WASP-Arp2/3 pathway to achieve nucleo-cytoplasmic transport and assembly of nucleocapsids.

There are intriguing evidences that host cytoskeletal proteins are involved in viral egress and assembly. Sodeik et al. (1997) provided compelling evidence that herpes virus simplex 1 parental nucleocapsids use microtubules for transport to the nucleus, and Cudmore et al. (1995) demonstrated that vaccinia virus progeny induce and use the vectoral polymerization of actin to bud from cells. It has become apparent that numerous viruses interact with actin at various stages throughout their life cycle, both disrupting and rearranging actin cytoskeleton to their advantage (Cudmore et al., 1997). P78/83 is conserved in most baculoviruses from lepidopteran insects suggesting that it maybe involved in some basic process of viral life cycle. Even characterization of *p*78/83 homologies has been carried out in group I baculoviruses, they are never characterized in group II baculoviruses.

A *p*78/83 homology, open reading frame 2 (*ha2*), is found in *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV) of the group II NPV (Chen et al., 2001). Its function remains elusive. In this report, we tried to characterize *ha*2 by transcriptional analysis, protein identification and localization. RT-PCR analysis indicated that *ha*2 was transcripted at the late stage of the infection. Three products with the sizes of 50, 46 and 35 kDa were detected in infected cells, implied that the different post-translation modifications might occurred. It might also be possible that the smaller proteins (46 and 35 kDa) arise from proteolytic degradation of the 50 kDa protein. However only the 50 kDa product was detected in both BV and ODV. We also provided evident that HA2 co-localized with cellular actin skeleton.

2. Materials and methods

2.1. Cells and virus

The *H. zea* cell line (BCIRL-HZ-AM1, HZ-AM1) and *Spodoptera frugiperda* (Sf21) cells were used and maintained at 28 °C in Grace's medium supplemented with 10% fetal bovine serum. The HaSNPV G4, whose genome has been entirely sequenced (Chen et al., 2001), and HaSNPV bacmid (HaHZ8) (Wang et al., 2003) were used as wild type (wt) virus and propagated in Hz-AM1 cells. An AcMNPV bacmid (bMON14272; Invitrogen) was also used.

2.2. Construction of plasmids and recombinant virus

The entire *ha*2 was amplified from the HaSNPV G4 genome with the primers: Ha2up 5′-*GAA TTC* **ATG** GTT CAA CTG CAA AGT GTT-3′ and Ha2down 5′-*GGA TCC* GCA ACT TGC GAT **TCA** GTT GAC AT-3′. The PCR product was first cloned into pGEM-T-Easy vector (Promega) (pT-HA2), and then cloned into pEGFP-N1 vector with *Eco*RI and *Bam*HI to generate the Ha2-egfp fusion gene. To get the recombinant virus, Ha2-egfp fusion gene was cloned into the vector pFastbac1 with *Eco*RI and *Not*I and produced the donor plasmid pFast-Ha2-egfp. The plasmid pFast-Ha2-egfp was then used to transformed competent DH10B cells containing helper and HaSNPV bacmid (HaHZ8) or AcMNPV bacmid (Invitrogen). The recombinant HaSNPV was used to transfect HzAM1 cells and AcMNPV bacmid DNA isolated from DH10B transfected Sf21 cells by Lipofectin (Invitrogen). Recombinant viruses vHa-Ha2-egfp, vAc-Ha2-egfp were harvested at 120 h post transfection (hpt). Recombinants were authenticated by PCR and restriction enzymes analysis. Virus titres were determined by end point dilution assay.

2.3. Preparation of antibody

Two primers, Ha2in (5′-*GGA TCC TTG* ***ATG*** *GAA CAA ATA CAG AAA GGA*-3′) and Ha2down (5′-*AAG CTT TTA AAC TTG CGA TTC AGT TGA CAT*-3′) were designed to amplify the truncated *Ha2* (corresponding to the C-terminal of Ha2: aa 201–413) from the pT-Ha2 and then cloned into the expression vector pGEX-KG with *Bam*HI and *Hin*dIII. This generated plasmid pGEX-cHa2, in which *ha2* is in-frame and fused with GST at the C terminus. *Escherichia coli* DH5αcells containing pGEX-cHa2 were grown to an OD_{600} of 0.4 and then induced with 1mM IPTG. After 3 h at 37 °C, cells were harvested and lysed with lysozyme, sonicated, and centrifuged at $5000 \times g$ for 10 min at 4 °C. The fusion protein, present in the pellet was separated in 12% SDS polyacrylamide gels and purified. Antiserum were generated by immunizing rabbits with purified protein (Sambrook et al., 1989) and tested by Western blot analysis. The antibody to OpMNPV ORF2 was kindly provided by Dr. G. F. Rohrmann (Department of Microbiology, Oregon State University).

2.4. Transcription analysis of ha2

HzAM1 cells (10^6) were infected with HaSNPV-G4 BV at a multiplicity of infection (MOI) of 5. Total RNA was isolated at 0, 4, 8, 16, 24, 48, 72 and 96 hpi by Trizol according to the manufacture's guidelines (GIBCO BRL). The RNA was dissolved in 50 μl water and quantified by absorbance measurement at 260 nm. Mock-infected HzAM1 cells were used as controls.

RT-PCR was performed using 1 μg total RNA as template for each time point. First strand cDNA synthesis was performed with AMV reverse transcriptase (Promega) and a 15-nt oligo-dT primer according to the manufacturer's instructions. The cDNA mixtures were amplified by PCR using the gene-specific primer, Ha2down (5′-GGA TCC GCA ACT TGC GAT TCA GTT GAC AT-3′) and an internal primer, Ha2in (5′-GGA TCC TTG ATG GAA CAA ATA CAG AAA GGA-3′). PCR products were analyzed on agarose gel.

2.5. Purification of HaSNPV BV and ODV fractions

Hz-AM1 cells were infected with BVs of HaSNPV G4 at an MOI of 0.1. After 3 days, the cell culture supernatant was collected and clarified at $2000 \times g$ for 10 min at 4 °C. The supernatant containing the BVs was passed through a 0.45 mm

pore-size filter. BVs in the filtrate were pelleted through a 25% (wt/wt) sucrose cushion in 0.1× TE (TE: 10 mM Tris/HCl, pH 7.5, and 1.0 mM EDTA) at 100,000 × *g* for 90 min at 4 °C, and resuspended in 0.1× TE. ODVs were purified from HaSNPV G4 polyhedra derived from infected *H. armigera* fourth-instar larvae (Chen et al., 2001).

2.6. Western blotting analysis

HzAM1 cells were infected with HaSNPV-G4 at a MOI of 5. Samples of total cell proteins were prepared from infected cells harvested at 0, 4, 8, 12, 24, 36, 48, 72 hpi. Protein samples were separated on 12% SDS-PAGE and transferred onto Hybond-N membrane (Amersham) by semi-dry electrophoresis (Ausubel et al., 1994). HA2-specific antiserum and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (Sino-American, China) were used as the primary and secondary antibodies, respectively. The signal was detected using a BCIP/NBT kit (Sino-American, China).

2.7. Fluorescence microscopy analysis

HzAM1 or Sf9 cells (1×10^5) were grown on glass cover slips in Petri dishes and infected with vHa-Ha2-egfp or vAc-Ha2-egfp at MOI of 10. At 24, 36, 48, 72 and 96 hpi, the cells were fixed and stained with Hoechst (Beyotime), then examined under a S2 Leica laser confocal scanning microscope for fluorescence. For the colocalization of HA2 and cellular actin, infected cells were fixed at 48 or 72 hpi for 5 min on ice in 2% formaldehyde and 0.2% glutaraldehyde in PBS, then washed once with PBS, solubilized with 0.15% Triton X-100 and stained with Rhordamine-Phalloidin (Sigma). vHa-egfp was used as GFP control.

2.8. Computer–assisted analysis

HA2 was analyzed using the ExPASy server (Appel et al., 1994) for the prediction of signal peptides, transmembrane regions and motifs. Protein comparisons with entries in the updated GenBank/EMBL, SWISS-PROT, and PIR databases were performed with BLASTP, FASTA and PSI-BLAST programs (Altschul et al., 1997). Multiple sequence alignments were performed with UW-GCG Pileup computer programs, with gap creation and extension penalties set to 8 and 2, respectively (Devereux et al., 1984). Alignment editing was done with Gendoc software.

2.9. Growth curves

For one-step growth curves, Hz-AM1 cells were infected with viruses (vHa-egfp and vHa-Ha2-egfp) at an MOI of 0.5. Supernatants were collected at the indicated times (0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120 hpi). The titers of supernatants were determined by end point dilution assay (EPDA) in Hz-AM1 cells, and the final results were checked 7 days after EPDA.

3. Results

3.1. Sequence analysis of ha2 and its homologues

Ha2 is located in the *Hin*dIII-A fragment of the HaSNPV genome (nt 735–1976 (Chen et al., 2001). Sequence analysis indicated that *ha2* is 1242 bp, and encodes a protein of 413 amino acids with a predicted molecular mass of 45.9 kDa. Two early transcription initiation motifs, TATA and CAGT, were found 238 and 138 nt upstream of the putative translational start site of *ha2*, respectively. A late baculoviral transcription initiation motif, ATAAG, was found 397 nt upstream of the putative translational start site, suggesting that *ha2* may be an early or late gene of HaSNPV. A T-rich sequence was found 63 nt downstream of the stop codon, which might function as the mRNA transcription termination and polyadenylation signal (Jin and Guarino, 2000).

Searches of databases showed that HA2 is conserved among all the lepidopteran 16 nucleopolyhedroviruses and 4 granuloviruses whose complete sequence have been reported, but not in baculoviruses from dipteran or hymenopteran hosts. Homology analysis indicated that HA2 is most closely related to HzSNPV ORF2 with 99% identity, consistent with the previous suggestion that HzSNPV and HaSNPV might be different strains of the same virus (Chen et al., 2002). Sequence alignments (data not show) indicated low sequence identity among all the HA2 homologues in NPV. However, the homologues are positionally conserved in their respective genomes and share conserved regions such as a proline rich region. Further analysis showed that HA2 contained common motifs of WASP or WASP-like protein such as RickA and ActA, including an acidic motif (A), a central basic motif or cofilin homology and WH2 motif. All of these proteins have the same motifs and organization. The motif conservation between HA2 and WASP proteins suggests that HA2 may be a functional homologue of WASP proteins and may have the ability to interact with host actin and could well be involved in virion transportation and/or assembly.

No signal peptide sequence was predicted on the putative HA2 protein by PSORT and SignalP software, and one strong transmembrane region from Gly218 to Leu236 was identified by TMpred software at the ExPASy server (Appel et al., 1994). In this 19 residues transmembrane domain, 17 are composed of highly hydrophobic alanine, glycine, leucine, isoleucine, serine and threonine. HA2 contained three potential N-linked glycosylation consensus sequences (N-X-S/T) at position 15, 151 and 278. Two of these, at amino acid position 15 and 278 were

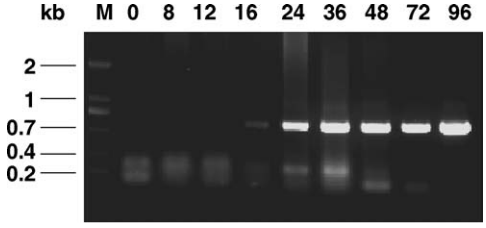

Fig. 1. Transcriptional analysis of *ha2*. Hz-AMI cells were infected with HaSNPV G4 at 5 MOI and harvested at 0, 8, 12, 16, 24, 36, 48, and 72 h post infection. RT-PCR was used to analyse *ha*2 transcription in each sample.

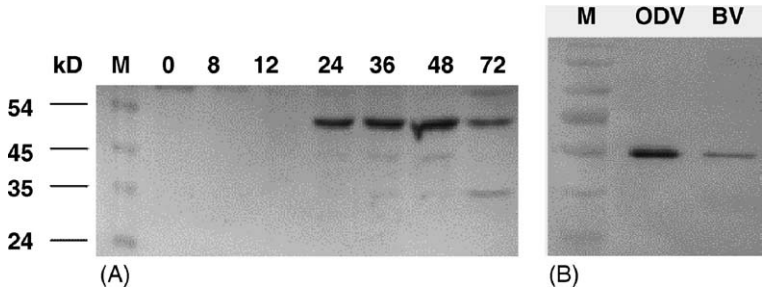

Fig. 2. *Ha2* expression analysis and detection in virions. 1×10^6 Hz-AMI cells were infected with HaSNPV G4 at MOI of 5. HA2 expression analysis with anti-HA2 antibody (A); HA2 detection in ODV and BV with anti-HA2 antibody (B).

predicted to be glycosylated by the NetNGlyc program of ExPASy server. Also there are numerous serine or theonine phosphorylation sites predicted by NetPhos program.

3.2. Transcription analysis of ha2

Transcription of *ha2* was examined by RT-PCR, using total RNA isolated from HzAM1 infected with HaSNPV at different time points. The transcript of *ha2* was first detected at 16 hpi and continued to be present until 96 hpi (Fig. 1), indicating that *ha2* is a late gene and may be transcribed from the late transcription initiation motif ATAAG.

3.3. Immunodetection of the HA2 protein in infected cells and in virus particles

Truncated HA2 (C terminal part, cHA2) was expressed in *E. coli* as a GST-cHA2 fusion protein and purified by SDS-PAGE. Specific antiserum was generated by immunized rabbits with the purified protein.

Western blot analysis of extracts of HaSNPV–infected Hz-AM1 cells revealed three specific bands in size of 35, 46 and 50 kDa (Fig. 2). The major band of 50 kDa was first detected at 24 hpi and peaked at 48 hpi, while the minor band of 46 kDa appeared at 24 hpi and was undetectable by 72 hpi. In contrast, the other minor 35 kDa was first observed at 48 hpi. These data are in agreement with the analysis of transcripts and indicate that HA2 was synthesized at a late stage of infection. The size of the 50 kDa protein is larger than the predicted 45.9 kDa based on its sequence, which suggests that this protein may be subject to post-translation modification. The 35 and 46 kDa forms might be a cleavage product at the late stage of infection. When antibody of OpMNPV ORF2 was used in the western blotting, only two bands in size of 46 and 35 kDa were detectable; the 50 kDa could not be detectable (data not shown.)

To investigate if HA2 is a structural component of HaSNPV virions, Western blot analysis of BV and ODV proteins was conducted. HA2 was detected in preparations of both ODVs and BVs (Fig. 2B), indicating that the protein is a structural component of ODVs ands BVs. However only the 50 kDa band was detected, the unmodified form of 46 kDa and truncated form of 35 kDa did not appear to be incorporated into the virus particles.

3.4. Cellular distribution of HA2 in HzAMI cells and Sf21 cells

The subcellular localization of the HA2 in permissive and non-permissive cells was investigated using a C-terminal *ha2-egfp* fusion construct. Two recombinant HaSNPVs were first generated by Bac-to-Bac systems. The *ha2-egfp* fusion gene was inserted into HaHZ8 and AcMNPV bacmid and produced vHa-Ha2-egfp and vAc-Ha2-egfp, respectively. Hz-AM1 cells were infected with vHa-Ha2-egfp and examined for fluorescence by

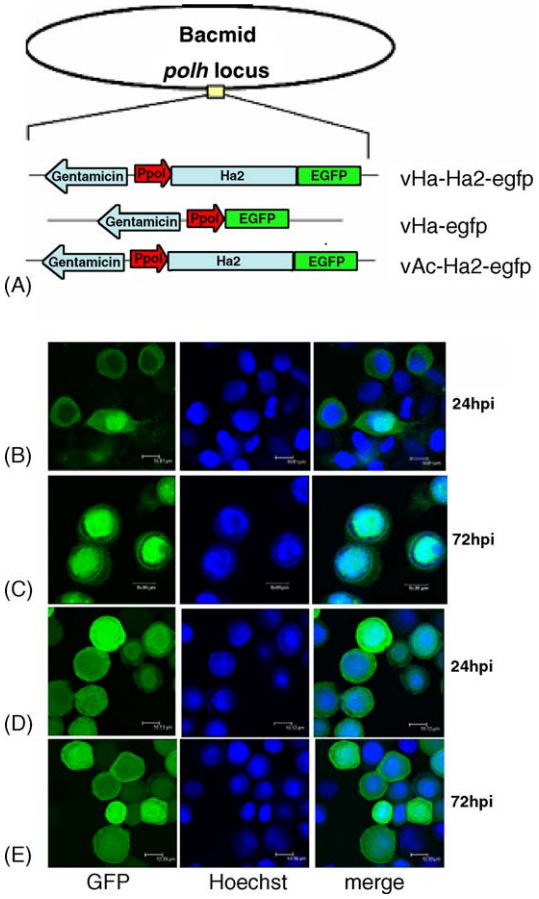

Fig. 3. HA2 distribution in Hz-AMI (B) and (C) and Sf9 (D) and (E). Schematic diagram of recombinant baculovirus constructs (A). Hz-AM1 were infected by vHa-Ha2-egfp (B) and (C) and Sf9 cells with vAc-Ha2-egfp (D) and (E) both at MOI of 10, fixed and stained with Hoechst and observed at 24 and 72 hpi.

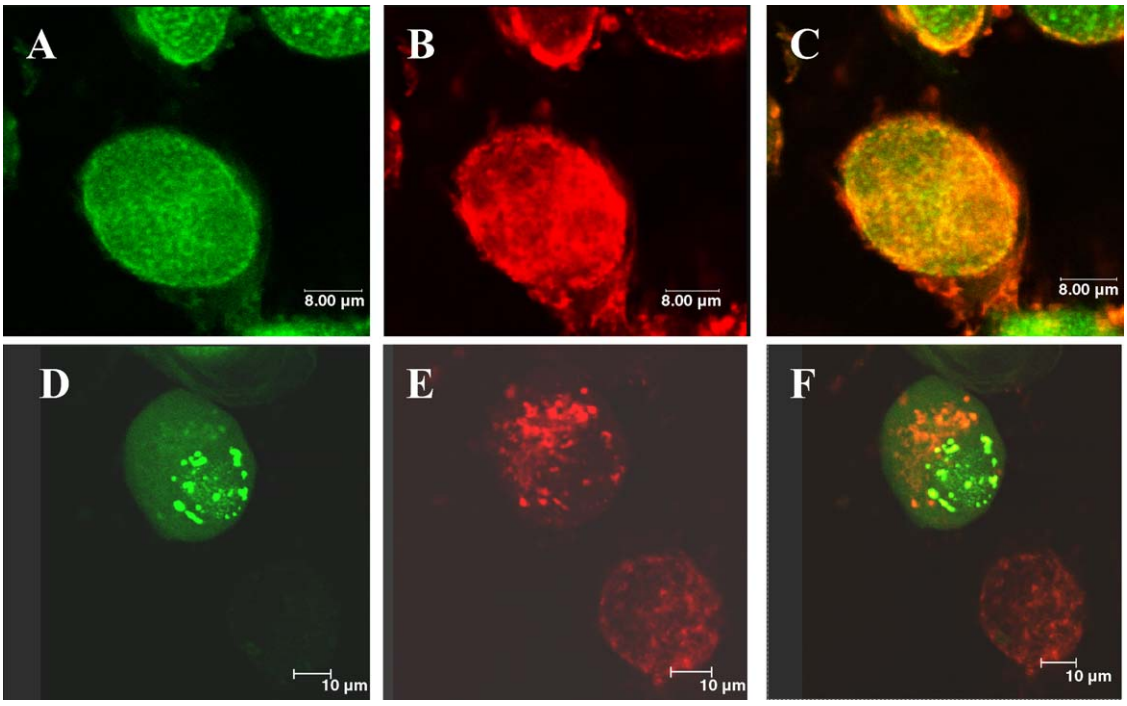

Fig. 4. Co-localization of HA2 and cellular actin. Hz-AMI cells were infected with vHa-Ha2-egfp at MOI = 5 without (A–C) or with 1 μg/ml cytochalasin D (D–F). (A) and (D), cells were stained with Rhordamin-Phalloidin at 48 hpi; (B) and (E), HA2-egfp distribution in cells at 48 hpi; (C) and (F), merge images.

confocal laser scanning microscopy at 24, 48, 72 and 96 hpi. The HA2-EGFP fusion protein was localized primarily in the cytoplasm in the early stage (Fig. 3B) and in the nucleus from 48 hpi (Fig. 3C) and had the tendency to form bright foci. However, when Sf9 cells infected with the vAc-Ha2-egfp, the fusion protein was localized both in the cytoplasm and in the nucleus (Fig. 3D and E). GFP alone showed homogeneous fluorescence in the cytoplasm and nucleus (data not show). The different localization of HA2 in Ha-AM1 and Sf21 might be due to the different cell lines and/or the different viruses, implied that host factor(s) and/or viral protein(s) might be involved.

3.5. Colocalization of HA2 and cellular actin

Hz-AM1 cells were infected with vHa-Ha2-egfp and vHa-egfp at 10 MOI. Infected cells were stained by Rhordamine-Phalloidin and then observed with laser confocal microscopy at 24, 36, 48, 72 and 96 hpi. HA2 was found to show co-localization with cell actin especially in late course of infection (Fig. 4A–C). Addition of cytochalasin D (1 μg/ml) to infected cells resulted in HA2 and actin aggregating in the nucleus and uncoupling the co-localization (Fig. 4D–F). It is in concord with the previous report that cytochalasin D treatment uncoupled P78/83 and F-actin co-localization, indicating that HA2 bounds to the pointed ends of actin filaments (Lanier and Volkman, 1998).

3.6. Biological assay of vHa-Ha2-egfp

The temporal kinetics of the one-step growth curve of budded virus of vHa-Ha2-egfp was similar to that of vHa-egfp (Fig. 5). Though the titer at 84 hpi of vHa-Ha2-egfp was about 0.15 logs

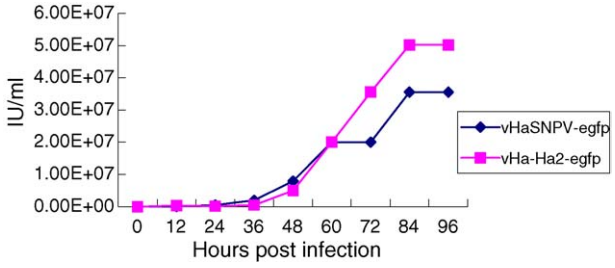

Fig. 5. One-step growth curves in Hz-AM1 cells. Cells were infected with vHa-egfp, vHa-Ha2-egfp at an MOI of 0.5. Progeny virus was assayed by the end point dilution assay (EPDA).

higher than that of vHaSNPV-egfp. The growth kinetics of both were quite similar. All titers increased to the highest at 84 hpi. Therefore, over-expression of HA2 did not appear to have an effect on the virus growth and, the recombinant virus vHa-Ha2-egfp has the same infectivity in HZ-AM1 cells.

4. Discussion

Ha2 is a conserved gene with homologues in most baculoviruses from lepidopteran insects. The length and the identity of the homologies are quite variable, but they share the same motifs of mammalian WASP proteins such as proline rich domain, acidic motif, cofilin domain and WH2 motif. Acidic motif (A), which was found to be Arp2/3 binding sequence and seems to increase the efficiency of actin nucleation as well as the parameter of motility (Machesky et al., 1999). Central basic motif termed cofilin was reported to be crucial to the stimulation of actin nucleation by Arp2/3 in vitro, and is also required for

actin polymerization and motility in cells (Miki and Takenawa, 2000). WH2 motif supplies the sequence of monomer-actin binding and it is essential for actin nucleation in vitro (Machesky et al., 1999). The domains and motif conservation between HA2 and the WASP family suggest that baculovirus might use the WASP–Arp2/3 pathway for assembly and egress. However, the *ha*2 homologue is not found in baculoviruses from *Diptera* or *Hymenoptera*. It is highly possible that viruses of *Lepidoptera* might have acquired the gene in the later evolution. Viruses of more ancient orders of *Diptera* and *Hymenoptera*, which only replicate in the midgut, must use a different mechanism in assembly and egress.

To elucidate *ha2* function, we analyzed the transcription and translation of *ha2* in HaSNPV-infected *H. armigera* cells, and investigated whether it was a structural component of the virions. RT-PCR showed that *ha2* transcription started at 16 hpi and continued until at least 96 hpi (Fig. 1) suggesting that *ha2* is a late gene whose transcription might be initiated from the ATAAG motif. We could not detect transcripts in the early stages of infection, even though the elements of early transcripts are present in *ha2* promoter.

Western blot analysis further confirmed that *ha2* is a late gene detected from 24 to 72 hpi (Fig. 2A). Also, multiple protein bands of HA2 with the size of 50, 46 and 35 kDa were detected in wild-type HaSNPV-infected Hz-AM1 cells. The 46 kDa protein is in good agreement with the predicted 45.9 kDa based on sequence, while the 50 and the 35 kDa have apparently been post translationally modified. Multiple phosphorylation sites in HA2 have been predicted by programs at ExPaSy server, so the 50 kDa band is likely to be a phosphorylated HA2. The AcMNPV P78/83 has two phosphorylation forms (Russell et al., 1997). Only the modified 50 kDa protein is incorporated into BVs and ODVs (Fig. 2B), the function of the 35 and 46 kDa products are under investigation.

We have found that HA2-EGFP co-localized with cellular actin skeleton as illustrated in Fig. 4. It has been demonstrated that the replication of either groups I or II nucleopolyhedrovirus is F-actin dependent. F-actin-dependent progeny morphogenesis appears to be a characteristic common among viruses in this genus that have lepidopteran hosts (Kasman and Volkman, 2000). Further, it is implicated that baculoviruses found to be F-actin dependent must encode gene products that specifically interact with and manipulate the actin cytoskeleton. In AcMNPV, P78/38 and P39 were found to bind actin in vitro and in vivo (Lanier and Volkman, 1998). A HA2-EGFP fusion co-localizes with actin and cytochalasin D treatment uncouples HA2-EGFP and F-actin colocalization, suggesting a possible association between the two and implying HA2 might have the ability to bind actin and induce actin polymerization, thus could be involved in the virion assembly and/or transportation.

Actin assembly plays an important role in many vital cellular processes, thus by recruiting actin, pathogens can quickly and successfully take over the host cell machinery to help in invasion and locomotion within and between cells. There are intriguing evidences that host cytoskeletal proteins are involved in viral assembly and egress. Sodeik et al. (1997) provided compelling evidence that parental nucleocapsids of herpes virus simplex 1 use microtubules for transport to the nucleus, and Cudmore et al. (1995) demonstrated that vaccinia virus progeny induced and used the vectoral polymerization of actin to bud out of cells. The vaccinia virus was also found to move along microtubles and recruit actin tails to support membrane protrusion, and thus facilitate cell to cell spread (Cudmore et al., 1995; Smith et al., 2003). Several other pathogens, including *Shigella flexneri* and *Listeria monocytogenes* and multiple Rickettia species have evolved to indirectly stimulate Arp2/3 complex-activated actin nucleation by activating WASP family members on their surfaces. Mimicking WASP or activation of the WASP family member leads to stimulation of the Arp2/3 complex and rapid actin assembly (Goldberg, 2001). It has become apparent that numerous pathogens interact with actin at various stages of their life cycle, both disrupting and rearranging actin cytoskeleton to their advantage (Cossart and Sansunetti, 2004; Cudmore et al., 1997; Gruenheid and Finley, 2003). Recently, it has been found that cortactin, an actin-binding protein and a pivotal regulator of the actin cytoskeleton, is the common target exploited by variety of microbial pathogens during infection. The protein cortactin has been shown to play a crucial role in invasion, actin-based motility, pedestal formation and cell scattering (Daly, 2004; Selbach and Backert, 2005; Weed and Parsons, 2001).

Whether the protein encoded by *ha*2 possess the ability to interact with Apr2/3 complex and to induce or accelerate actin polymerization like WASP proteins is yet to be determined. We are interested in how HaSNPV use the protein to recruit factors for actin polymerization and how the protein interacts with cortactin to manipulate the actin networks. These are intriguing areas in baculovirus replication and further experiments shall be designed and conducted to elucidate them.

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