The F protein of *Helicoverpa armigera* single nucleopolyhedrovirus can be substituted functionally with its homologue from *Spodoptera exigua* multiple nucleopolyhedrovirus

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F proteins of group II nucleopolyhedroviruses (NPVs) are envelope fusion proteins essential for virus entry and egress. An F-null *Helicoverpa armigera* single nucleocapsid NPV (HearNPV) bacmid, HaBacΔF, was constructed. This bacmid could not produce infectious budded virus (BV) when transfected into HzAM1 cells, showing that F protein is essential for cell-to-cell transmission of BVs. When HaBacΔF was pseudotyped with the homologous F protein (HaBacΔF-HaF, positive control) or with the heterologous F protein from *Spodoptera exigua* multinucleocapsid NPV (SeMNPV) (HaBacΔF-SeF), infectious BVs were produced with similar kinetics. In the late phase of infection, the BV titre of HaBacΔF-SeF virus was about ten times lower than that of HaBacΔF-HaF virus. Both pseudotyped viruses were able to fuse HzAM1 cells in a similar fashion. The F proteins of both HearNPV and SeMNPV were completely cleaved into F1 and F2 in the BVs of vHaBacΔF-HaF and vHaBacΔF-SeF, respectively, but the cleavage of SeF in vHaBacΔF-SeF-infected HzAM1 cells was incomplete, explaining the lower BV titre of vHaBacΔF-SeF. Polyclonal antisera against HaF1 and SeF1 specifically neutralized the infection of vHaBacΔF-HaF and vHaBacΔF-SeF, respectively. HaF1 antiserum showed some cross-neutralization with vHaBacΔF-SeF. These results demonstrate that group II NPV F proteins can be functionally replaced with a homologue of other group II NPVs, suggesting that the interaction of F with other viral or host proteins is not absolutely species-specific.

**INTRODUCTION**

The initial infection events of enveloped viruses are usually mediated by their envelope proteins. The envelope fusion proteins (EFPs) of baculoviruses play important roles in several steps in the infection processes, such as receptor binding, fusion with endosomal membrane and efficient budding (Hefferon et al., 1999; Kingsley et al., 1999; Oomens & Blissard, 1999). There are two types of EFP in baculoviruses: GP64 and F protein. GP64 is a glycoprotein encoded by group I nucleopolyhedroviruses (NPVs), such as *Autographa californica* multinucleocapsid NPV (AcMNPV) (Ayres et al., 1994), *Orgyia pseudotsugata* MNPV (OpMNPV) (Ahrens et al., 1997) and the *Bombyx mori* NPV (Gomi et al., 1999). F protein has been identified in group II NPVs such as *Lymatia dispar* MNPV (Pearson et al., 2000), *Spodoptera exigua* MNPV (SeMNPV) (Ijkel et al., 2000) and *Helicoverpa armigera* single nucleocapsid NPV (HearNPV) (Long et al., 2006a). F homologues have been found in all baculovirus genomes sequenced so far.

GP64 and F proteins differ in their structure and mode of action. In contrast to the high amino acid identity among GP64 proteins (>74%), F proteins are more diverse (20–40%) (Pearson et al., 2000). Phylogenetic analysis has suggested that F proteins may be an ancient type of EFPs (Pearson et al., 2000). In group I NPVs, there is a truncated form of F, which occurs in the budded virus (BV) phenotype, but is not responsible for fusion (Lung et al., 2003). It is essential for F proteins to be cleaved by cellular furin for activation, which generates a membrane-anchored F1 subunit and membrane-distal F2 subunit connected by a disulfide bond (Ijkel et al., 2000; Westenberg et al., 2002). GP64 does not require furin cleavage for activation. Furthermore, disulfide bonds are not involved in the formation of F protein oligomers, as is the case for the GP64 trimeric structure (Long et al., 2006a; Oomens et al., 1995).
So far, several F proteins of group II NPVs have been demonstrated to be able to replace GP64 by pseudotyping AcMNPV (Long et al., 2006a; Lung et al., 2002). However, the reverse does not occur (Westenberg & Vlak, 2008). In this report, we addressed this issue by replacing the F protein of HearNPV (HaF) with its homologue from SeMNPV (SeF). HaF and SeF are the well-studied representatives of baculoviral F proteins (Ijkel et al., 2000; Long et al., 2006a) and are typical class I fusion proteins. HaF and SeF use the same insect-cell receptor, whereas GP64 uses a different receptor (Westenberg et al., 2007). The amino acid identity of SeF and HaF is about 33% but the two proteins have many common structural features such as an N-terminal signal peptide, furin cleavage site, fusion peptide region, transmembrane region (TM) and a cytoplasmic tail domain (CTD) (Ijkel et al., 2000; Long et al., 2006b).

In this report, we describe the construction and testing of a HaF-knockout HearNPV bacmid, HaBacAF. HaF and SeF were inserted into this HaF-null HearNPV bacmid, generating HaBacAF-HaF (rescue control) and HaBacAF-SeF, respectively. Transfection/infection assays and one-step growth curves were conducted to compare the BV production of HaBacAF-HaF virus (vHaBacAF-HaF) and vHaBacAF-SeF. Low-pH-dependent membrane fusion assays, Western blot analysis and neutralization assays were performed to investigate the characteristics of the pseudotyped viruses and hence the function of F in a heterologous group II NPV.

**METHODS**

**Insect cells and virus.** HzAM1 cells were maintained at 27 °C in Grace’s insect medium (Gibco-BRL) supplemented with 10% fetal bovine serum (FBS) (pH 6.0). The HearNPV bacmid (HaBacH28) used in this study was constructed previously in our laboratory (Wang et al., 2003).

**Construction of recombinant viruses.** The HaF gene of HaBacH28 was knocked out by homologous recombination in recombinant *E. coli* BJ5183 cells according to the method of Hou et al. (2002), replacing the HaF gene by the tetracycline-resistance gene. Briefly, 1.1 kb of sequence upstream of the HaF gene was amplified by PCR with primers HaF-up-for (5'-GGTCACATTTCACAAATTCATGTCGGG-3'; *Kpn*I site underlined) and HaF-up-rev (5'-AAGCTTACATGTCGGGGAATTCGTTGGAACAC-3'; *HindIII* site underlined). An 850 bp sequence downstream of the HaF gene was obtained with primers HaF-down-for (5'-AAGCTTGAGTCGTCTTTCTGC-3'; *Sad*I site underlined) and HaF-down-rev (5'-GAAGTCTCGTACACACACTGATATTGC-3'; *EcoRI* site underlined) using HearNPV as a template. The PCR products were cloned into a pKS vector (Stratagene). An enhanced green fluorescent protein gene (*egfp*) under the control of the hsv2 promoter was further cloned into the pKS vector using the *Xho*I and *HindIII* sites. A tetracycline-resistance gene (Tes*) was amplified to replace the *Tet* gene of pBacAF. This transfer vector was digested by *Kpn*I and *EcoRI* and the linear fragment containing *Tes*, *egfp* and the HaF gene flanking sequences was used to transform BJ5183 competent cells containing HaBacH28 and helper plasmid pKD46 as described by Hou et al. (2002). Positive clones were selected by tetracycline and kanamycin resistance. The correct bacmid clone was authenticated by PCR and named HaBacAF.

For the construction of pseudotyping viruses, the OpMNPV gp64 promoter (Op166) was digested from p166BRNX-AcV5 (Ijkel et al., 2000) using *Sac*I and *EcoRI* and cloned into pUC19 (TaKaRa) to give pUC19-Op166. The Op166 promoter was then excised by *BamHI* digestion and subcloned into the transfer vector pFastBac1 (Bac-to-Bac Baculovirus Expression System; Gibco-BRL), generating pFB-Op166. The HaF gene was amplified from the HearNPV DNA template by PCR with primers HaF-for (5'-AAGCTTATGTTGC-GCCGATTTAATTGATG-3'; *HindIII* site underlined) and HaF-rev (5'-GGATCCAGCTCGTTAGGATTGTTCGTCG-3'; *HindIII* site underlined). The PCR product was digested with *HindIII* and cloned into the transfer vector pFB-Op166, generating pFB-Op166-HaF. The SeF gene was excised from p166AcV5-SeF (Ijkel et al., 2000) by *BamHI* and *EcoRI* digestion and cloned into *BamHI/EcoRI*-cleaved pFastBac1, generating pFB-SeF. The Op166 promoter was digested from pUC19-Op166 by *BamHI* and cloned into pFB-SeF, generating pFB-Op166-SeF, pFB-Op166-HaF or pFB-Op166-SeF was used to co-transform DH10Bac cells with a HaBacAF bacmid and the helper plasmid expressing transposase (Bac-to-Bac Baculovirus Expression System; Gibco-BRL). Recombinant bacmids were selected by gentamicin resistance and blue/white screening. Correct recombinant bacmids were identified by PCR with M13 primers and *EcoRI* digestion, and named HaBacAF-HaF and HaBacAF-SeF, respectively.

**Transfection and infection assays.** HzAM1 cells were inoculated into 35 mm diameter tissue culture dishes at a density of 5 x 10⁵ cells per dish. After 2 h, cells were transfected with 1 μg recombinant viral DNA (HaBacAF, HaBacAF-HaF or HaBacAF-SeF) using 15 μl Lipofectin according to the Bac-to-Bac Expression Systems manual (Invitrogen). For the infection assay, at 6 days post-transfection (p.t.), 1 ml supernatant from the transfection was centrifuged at 956 g for 5 min to remove cell debris, and supernatants were used to infect HzAM1 cells. Cells were monitored by fluorescence microscopy at 72 h p.t. or post-infection (p.i.).

**One-step virus growth curves.** HzAM1 cells were infected with vHaBacAF-SeF or vHaBacAF-HaF at an m.o.i. of 10 TCID₅₀ units per cell. At 0, 12, 24, 48 and 72 h p.i., supernatants were harvested and titrated by an end-point dilution assay. Each virus infection was done in triplicate. BV titres were log-transformed and statistically analysed with two-way analysis of variance in SPSS (SPSS Inc., 2003) with virus type and time as factors.

**Low-pH induced envelope fusion assay.** The syncytium-forming ability of the pseudotyped viruses was tested according to the method of Blissard & Wenz (1992) with a slight modification. Briefly, HzAM1 cells were infected by recombinant vHaBacAF-HaF or vHaBacAF-SeF at an m.o.i. of 10 TCID₅₀ units per cell. At 24 h p.i., the cells were washed twice with Grace’s insect medium and then treated with acidic (pH 5.0) Grace’s insect medium. After being exposed to the low-pH medium for 5 min, the cells were further cultured with normal Grace’s insect medium containing 10% FBS. Syncytium formation was observed under a fluorescence microscope at 24 h after the downward pH shift.

**Western blot analysis of recombinant BVs and infected cells.** For Western blot analysis, anti-HaF1 and anti-SeF1 antibodies were used. To generate anti-HaF1 antibody, the HaF1 sequence fragment was inserted into a pKS vector (Stratagene). An enhanced green fluorescent protein gene (*egfp*) under the control of the hsv2 promoter was fused to the C-terminus of the HaF1 gene. The recombinant plasmid was transformed into E. coli to express the fusion protein. The fusion protein was purified by nickel chelation chromatography and used for Western blot analysis. For the infection assay, at 6 days post-transfection (p.t.), 1 ml supernatant from the transfection was centrifuged at 956 g for 5 min to remove cell debris, and supernatants were used to infect HzAM1 cells. Cells were monitored by fluorescence microscopy at 72 h p.t. or post-infection (p.i.).
cloned into a pET28a expression vector (Novagen). To generate anti-ScF antibody, an ScF sequence fragment without the TM region was amplified by PCR using forward primer 5'-GGGGGATCCTGGGGCTTATTTAATTTGCGAC-3' (BamHI site underlined) and reverse primer 5'-GGGGAATTCCTCTTTTCTCTGTAATGAAATGCCTACC-3' (HindIII site underlined), and cloned into a pET28a expression vector. The expression plasmids containing HaF1 and SeF1 were transformed into BL21 cells and the proteins were induced with 1 mM IPTG at 37°C for 3 h. The expressed HaF1 and SeF1 proteins were purified by continuous-elution electrophoresis using a Model 491 Prep Cell (Bio-Rad) and used to immunize rabbits to generate polyclonal antisera against these proteins.

Western blot analysis was performed to detect furin cleavage of F proteins in the recombinant BVs as well as in the infected cells. HzAM1 cells were infected with recombinant vHaBacF-HaF or vHaBacAF-SeF at an m.o.i. of 10 TCID50 units per cell, and SeUCR cells were infected by SeMNPV at the same m.o.i. At 3 days p.i., 2 ml supernatant containing fresh BVs was harvested and centrifuged at 956 g for 5 min to remove cell debris and then at 20800 g for 30 min at 4°C. The sedimented BVs and infected-cell samples were disrupted in 6× SDS-PAGE sample buffer and separated by 12% SDS-PAGE. The proteins in the gel were transferred to Hybond-N membranes (Amersham) by semi-dry electrophoresis. Western blot analysis was performed with polyclonal anti-HaF1 or anti-SeF1 antiserum together with Grace's insect medium containing NBT/BCIP (SABC). Antibody and alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin as secondary antibody; the signals were detected with NBT/BCIP (SABC).

Neutralization assays. HzAM1 cells were inoculated into 24-well tissue culture plates at a density of 2 × 10^5 cells per well. vHaBacAF-HaF or vHaBacAF-SeF (m.o.i. of 1 TCID50 unit per cell) was incubated with different amounts of polyclonal rabbit anti-HaF1 or anti-SeF1 antiserum together with Grace's insect medium containing 10% FBS to a final volume of 300 μl. For mock neutralization, only virus (vHaBacAF-HaF or vHaBacAF-SeF; m.o.i. of 1 TCID50 unit per cell) was incubated with Grace's insect medium with 10% FBS to a volume of 300 μl. The viruses were also incubated with pre-immune serum as controls. All of the mixtures were incubated for 1 h at room temperature and then added to monolayers in 24-well tissue culture plates for 1 h to allow virus attachment. The mixtures were then replaced with normal medium and infection rates were quantified at 60 h.p.i. using a Beckman Coulter (EPICS XL) flow cytometer.

RESULTS

Generation of HaF-knockout HearNPV bacmid and pseudotyped bacmids

The HaF-knockout bacmid HaBacAF and pseudotyped bacmids HaBacAF-HaF and HaBacAF-SeF were generated as described in Methods; their genomic organization is shown in Fig. 1(a). Positive clones were confirmed by PCR amplifications (data not shown) and EcoRI analysis (Fig. 1b). In HaBacHz8, the HaF gene is located in an 8.9 kb EcoRI fragment. In HaBacAF, the 8.9 kb band was changed to a 9.5 kb band due to substitution of the HaF gene with a TetR and eGFP cassette (Fig. 1b). In comparison with HaBacAF, the EcoRI profile of HaBacAF-HaF contained additional 14.4 kb and 2.7 kb bands, but did not have the 12.2 kb band; these changes were due to insertion of the HaF gene (Fig. 1b). In HaBacAF-SeF due to insertion of the SeF gene, a new band of 17.1 kb replaced the band of 12.2 kb in HaBacAF (Fig. 1b). All of the bands of mutant bacmids were shifted as expected (indicated by arrowheads, Fig. 1b), confirming that the bacmid constructs were correct.

Transfection and infection assays

HzAM1 cells were transfected with recombinant bacmid HaBacAF, HaBacAF-HaF and HaBacAF-SeF, and their parent bacmid HaBacHz8. (b) Restriction enzyme analysis of the parental and recombinant bacmids. Recombinant bacmid DNAs were isolated and analysed by EcoR I digestion. Shifted bands are indicated by arrowheads (original bands with open arrowheads; shifted bands with filled arrowheads).
vHaBacΔF-SeF contained infectious virus (Fig. 2e, f). The results thus showed that when HaF was eliminated from HearNPV, no infectious BV was generated (Fig. 2d), whilst both HaF and SeF rescued the infectivity of HearNPV (Fig. 2e, f). Therefore, HaF is an essential gene for both HaF and SeF rescued the infectivity of HearNPV, no infectious BV was generated (Fig. 2d), whilst at 72 h p.i., the BV titre of vHaBacΔF-HaF was 1.70 ± 0.17 x 10^6 TCID50 units ml^-1 and the BV titre of vHaBacΔF-SeF was 1.00 ± 0.17 x 10^6 TCID50 units ml^-1, whilst at 72 h p.i., the BV titre of vHaBacΔF-HaF was 1.24 ± 0.00 x 10^0 TCID50 units ml^-1 and the BV titre of vHaBacΔF-SeF was 1.70 ± 0.05 x 10^0 TCID50 units ml^-1. Therefore, in the late phase of infection, the infectious BV production of vHaBacΔF-HaF was almost ten times that of vHaBacΔF-SeF. Statistical analysis showed that vHaBacΔF-SeF had a significantly decreased BV titre compared with the rescued virus vHaBacΔF-HaF (F = 92.375, d.f. = 1, 19, P < 0.01).

Syncytium formation assay of the pseudotyped viruses
As baculovirus F proteins are mildly acid-triggered membrane fusion proteins and cause low-pH-dependent membrane fusion, an assay was conducted to examine the expression and fusogenic ability of both F proteins (Fig. 4). As indicated by arrows in Fig. 4(c) and (d), multinuclear cells were detected in both vHaBacΔF-HaF- and vHaBacΔF-SeF-infected HzAM1 cells at 24 h after the pH was shifted down. Observation of the fusion level (number of syncytia) and the size of syncytia showed the fusion ability of vHaBacΔF-SeF to be similar to that of vHaBacΔF-HaF in infected HzAM1 cells.

Western blot analyses of the recombinant BVs and infected cells
To detect the F proteins in the recombinant BVs, Western blot analyses were conducted. Fig. 5(a) shows that the anti-HaF1 antiserum detected the expected 59 kDa band for SeF (Ijkel et al., 2000) in vHaBacΔF-HaF BVs but not in vHaBacΔF-SeF BVs; the anti-SeF1 antiserum detected the expected 60 kDa band for SeF (Ijkel et al., 2000) in vHaBacΔF-SeF BVs but not in vHaBacΔF-HaF BVs. Antiserum against HearNPV nucleocapsid protein VP80 served as a control for equal amounts of BV protein on the blot. The results indicated that both HaF and SeF were correctly cleaved into subunits F1 and F2 incorporated into the BVs, as no F0 bands were detected (Fig. 5a).

Western blot analysis was also used to detect the expression and cleavage of F proteins in infected cells (Fig. 5b). The results showed that, in vHaBacΔF-HaF-infected HzAM1 cells, a large amount of HaF was cleaved by furin, yielding a major F1 subunit band of ~59 kDa. There was still a minor band of uncleaved HaF0 (80 kDa). In comparison, the
cleavage of SeF in HzAM1 cells was not as complete, with only a small proportion of the SeF protein being processed into the F₁ subunit (~60 kDa), leaving a large amount of uncleaved SeF₀ (80 kDa). This was quite different from the situation with SeMNPV in its host cells (SeUCR cells), where most of the SeF was cleaved (Fig. 5b). Interestingly, we observed that, although a large proportion of SeF remained uncleaved in HzAM1 cells, the F protein incorporated into the surface of pseudotyped vHaBacAF-SeF BVs appeared to be cleaved (Fig. 5a).

Neutralization assays of pseudotyped viruses with specific and non-specific antisera

To test further the functionality of SeF relative to HaF, neutralization assays were carried out using monoclonal antibodies against SeF₁ and HaF₁ and using vHaBacAF-HaF and vHaBacAF-SeF (m.o.i. of 1 TCID₅₀ unit ml⁻¹) (Fig. 6). Anti-HaF₁ (Fig. 6a) and anti-SeF₁ (Fig. 6b) antisera specifically neutralized the infectivity of vHaBacAF-HaF and vHaBacAF-SeF, respectively, in a dose-dependent manner. The neutralizations were highly efficient, in that with 25 µl anti-HaF₁ antiserum or 1 µl anti-SeF₁ antiserum, more than 98% infection by vHaBacAF-HaF or more than 99% by vHaBacAF-SeF infection, respectively, was neutralized. The same amounts of pre-immune sera had no effect on virus infection compared with the virus-alone controls (data not shown). Anti-HaF₁ antiserum also showed some cross-neutralization of vHaBacAF-SeF: 50% inhibition of infection was achieved with 25 µl anti-HaF₁ antiserum (Fig. 6a). The results suggest that both SeF₁ and HaF₁ subunits contain neutralizing epitopes and that some of the epitopes may have similar structures.

DISCUSSION

The F proteins of baculoviruses are structurally more diverse than the GP64 proteins (Pearson et al., 2000). For HaF and SeF, the amino acid identity is relatively low (~33%) and the question is whether this difference precludes the functionality of a heterologous F into group II NPVs. For this purpose, we successfully generated a group II F knockout bacmid (HaBacAF; Fig. 1) and showed that F is essential for cell-to-cell transmission of infection. Our results also demonstrated that vHaBacAF-SeF produced infectious BVs with kinetics similar to those of vHaBacAF-HaF in HzAM1 cells, and that the fusion ability of these two pseudotyped viruses was similar. The successful substitution of HaF by SeF showed experimentally that F proteins are functional analogues in a group II NPV background. Despite low amino acid identity, the assembly of BVs was not compromised. This suggests a low degree of specificity of F in BV assembly in a group II NPV context. It has already been shown that F proteins of group II NPVs can pseudotype group I NPVs lacking GP64 (Lung et al., 2002). We have demonstrated here that type II F proteins can also pseudotype other F-null type II NPVs.

One-step growth curve analysis (Fig. 3) showed that the infectious BV production of vHaBacAF-SeF was lower than that of vHaBacAF-HaF, in which the F function was rescued. This observation might be explained by the incomplete cleavage of SeF in HzAM1 cells. The F protein is expressed as a precursor that undergoes cleavage by a pro-protein convertase (furin) of the host for activation (Ikel et al., 2000; Westenberg et al., 2002). In this respect, the processing of F proteins occurs in a fashion similar to the EFPs of Paramyxoviridae, Orthomyxoviridae, Togoviridae, Retroviridae and Herpesviridae (Lazarowitz et al., 1971; Meyer et al., 1990; Morse et al., 1992; Scheid & Choppin, 1977; Veronese et al., 1985). The furin enzyme is located in the trans-Golgi network and cleaves the EFPs in a virus-independent manner at the R-X-R/K-R motif (Hosaka et al., 1991; Vey et al., 1994). In vHaBacAF-SeF-infected HzAM1 cells, furin cleavage of SeF was not as complete as cleavage of HaF in vHaBacAF-HaF-infected cells (Fig. 5b). However, it was difficult to detect uncleaved SeF₀ present in the BVs of HaBacAF-SeF (Fig. 5a).

It has been reported that in the human immunodeficiency virus (HIV) infection process, although uncleaved forms of gp160 (HIV-1) or gp140 (HIV-2) are extensively accessible at the cell surface, the precursor is not incorporated into virus particles following budding from the cell surface (Mouลาด et al., 1999). The high proportion of uncleaved gp160 was explained by a large amount of glycoprotein retained in the endoplasmic reticulum (ER), and only a
minority of the gp160 molecules reach the trans-Golgi compartment for furin cleavage (Hallenberger et al., 1992). Therefore, the incomplete cleavage of SeF may be due to SeF being retained in the ER of HzAM1 cells with only relatively few molecules of SeF reaching the trans-Golgi to be cleaved. The incomplete cleavage of SeF may also be due to the fact that HzAM1 cells were used, which is not a normal host cell for SeMNPV. It is possible that the furin in HzAM1 cells recognizes the RNKR motif of HaF1 more efficiently than the RSKR motif of SeF. As only cleaved SeF protein was incorporated into HaBacΔF-SeF BV particles, the lower titre of vHaBacΔF-SeF BV particles may be explained by a lower amount of correctly processed SeF proteins available on the cell membrane of HzAM1 cells for efficient viral budding. The different processing efficiency of the F proteins needs to be investigated further, and may reveal a process important for host-range determination. In this report, however, we did not investigate whether the lower infectious BV production of vHaBacΔF-SeF was due to lower BV production or to lower infectivity per BV. Further experiments such as real-time quantitative PCR will be carried out to answer this question.

Antisera against virus EFPs can neutralize infection. Therefore, we tested the neutralizing ability of polyclonal antibodies against HaF1 and SeF1. The F1 subunit of F proteins includes the fusion peptide, the predicted heptad repeats, and the TM and CTD regions. The N-terminal peptide in F1 and the heptad repeats have been proven to be functional as fusion and oligomerization regions, respectively (Long et al., 2006a; Westenberg et al., 2004). The antisera against SeF1 and HaF1 had specific neutralizing effects against the pseudotyped virus harbouring their respective F protein, suggesting there are neutralizing epitopes in F1 subunits. A similar situation exists with paramyxoviruses and HIV-1, where several neutralizing epitopes in paramyxovirus F1 domains and HIV-1 gp41 have been identified (Toyoda et al., 1988; Zolla-Pazner et al., 1999). The antiserum against the HaF1 domain also had cross-neutralizing effects on vHaBacΔF-SeF, suggesting some conformational similarities between the two F proteins.

The results described in this paper suggest that the interactions of baculovirus F orthologues with other viral or host proteins are not species-specific, at least in the case of HaF and SeF. It remains to be seen whether this holds true for all F proteins or all group II viruses, in particular those that are more distantly related. It was suggested that the CTD of F, which was dispensable in pseudotyping
The availability of HaBac (2008). This can now also be tested experimentally through homologous HearNPV F protein (Westenberg & Vlak, 2008). Recently, a report revealed that GP64 alone failed to insert in the presence of the group II NPVs in mammalian cells (Liang et al., 2005). However, this insertion was in the presence of the homologous HearNPV F protein (Westenberg & Vlak, 2008). Recently, a report revealed that GP64 alone failed to pseudotype an F-null SeMNPV (Westenberg & Vlak, 2008). This can now also be tested experimentally through the availability of HaBacΔF (Fig. 1). Such investigations will help us to understand the similarity and differences between GP64 and F protein functioning.

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REFERENCES


Fig. 6. Neutralization assays of pseudotyped viruses. vHaBacAF-HaF or vHaBacAF-SeF was incubated with increasing amounts of anti-HaF (a) or anti-SeF (b) antibodies for 1 h at room temperature. The mixture of virus and antiserum was used to infect HzAM1 cells (m.o.i. of 1 TCID50 unit per cell). The infection rate of HaBacAF-HaF (hatched bars) and HaBacAF-SeF (open bars) were quantified at 60 h post-infection (p.i) by flow cytometry. Results are shown as means ± SEM.

AcMNPV, may be involved in a specific interaction with group II BV proteins (Long et al., 2006b). Our data suggest that these interacting proteins, if there are any, should be able to cross-interact with CTDs of different group II F proteins, at least in the case of SeMNPV and HearNPV. It has been shown that GP64 can be expressed on the surface of HearNPV BV and hence can promote the transduction of group II NPVs in mammalian cells (Liang et al., 2005). However, this insertion was in the presence of the homologous HearNPV F protein (Westenberg & Vlak, 2008). Recently, a report revealed that GP64 alone failed to pseudotype an F-null SeMNPV (Westenberg & Vlak, 2008). This can now also be tested experimentally through the availability of HaBacΔF (Fig. 1). Such investigations...
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