Baculovirus envelope fusion proteins F and GP64 exploit distinct receptors to gain entry into cultured insect cells

Marcel Westenberg, Peter Uijtdewilligen† and Just M. Vlak

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

Group II nucleopolyhedroviruses (NPVs), e.g. Helicoverpa armigera (Hear) NPV and Spodoptera exigua (Se) MNPV (multiple NPV), lack a GP64-like protein that is present in group I NPVs, e.g. Autographa californica (Ac)MNPV, but have an unrelated envelope fusion protein named F. Three AcMNPV viruses were constructed by introducing AcMNPV gp64, HearNPV f or SeMNPV f genes, respectively, into a gp64-negative AcMNPV bacmid. S21 cells were incubated with different amounts of inactivated budded virus to occupy receptors and were subsequently infected with a fixed amount of infectious virus to compete for attachment. The results suggest that GP64 and F act on their own and use different receptors, while the two different F proteins exploit the same receptor. Additionally, gp64-null AcMNPV pseudotyped with baculovirus F was, in contrast to GP64, unable to transduce mammalian cells, indicating that mammalian cells do not possess baculovirus F protein receptors despite the structural similarity of baculovirus F to vertebrate viral fusion proteins.

In general, host and tissue tropism of viruses is often determined by the receptor they use for their attachment to cells. The host range of baculoviruses differs between species. For instance, AcMNPV is able to infect at least 27 insect species (Adams & McClintock, 1991), whereas Spodoptera exigua (Se)MNPV can only infect the beet armyworm Spodoptera exigua (Onstad, 2007). However, this difference in host range is probably not only related to their type of envelope fusion protein. SeMNPV for instance is capable of transducing a variety of non-permissive cells originating from different insect species (Yanase et al., 1998). Nevertheless, Wickham et al. (1992) showed, by means of competition experiments, that the baculoviruses AcMNPV and Lymantria dispar (Ld)MNPV, with respectively a GP64 and F protein, use different insect cell receptors. On the other hand, Hefferon et al. (1999) showed in a similar setup that AcMNPV and Orgyia pseudotsugata (Op)MNPV, both containing GP64, use the same insect cell receptor. However, these experiments do not give direct evidence that the different receptor usage of AcMNPV and LdMNPV is directly related to the type of envelope fusion protein. In AcMNPV and LdMNPV there are 75 genes, which are only present in one of the two genomes (Ayres et al., 1994; Kuzio et al., 1999). One or more of these genes might encode a protein, which contributes to the different receptor usage.

To investigate experimentally whether the envelope fusion protein is solely responsible for the attachment, two near-isogenic recombinant AcMNPV viruses, vAcgp64−/Acgp64...

The Baculoviridae are a family of large, enveloped, double-stranded DNA viruses that are exclusively pathogenic for arthropods, predominantly insects of the order Lepidoptera (Adams & McClintock, 1991). Baculoviruses are classified into two genera, Nucleopolyhedrovirus (NPV) and Granulovirus (GV). The NPVs can be phylogenetically subdivided into group I and II NPVs (Bulach et al., 1999). The NPVs contain stranded DNA viruses that are exclusively pathogenic and are subdivided into group I NPVs, e.g. Baculoviridae The Journal of General Virology (2007), 88, 3302–3306 DOI 10.1099/vir.0.83240-0

†Present address: Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Geert Grootplein 28, 6525 GA Nijmegen, The Netherlands.
and vAcgp64−/HaF, were used (Long et al., 2006; Lung et al., 2002). These viruses only differ in their type of envelope fusion protein, AcMNPV GP64 or Helicoverpa armigera (Hear)NPV F protein, respectively. These viruses have been made by Tn7 transposition of an expression cassette, containing the p6.9 promoter-GUS reporter and the AcMNPV gp64 gene or the HearNPV f gene under the control of the AcMNPV gp64 promoter, in the polyhedrin locus of an AcMNPV bacmid in which the original gp64 gene was replaced by a chloramphenicol acetyl transferase (cat) gene (Fig. 1a). These bacmids were transfected into Sf21 cells (Vaughn et al., 1977) in order to generate infectious BVs as described previously (Westenberg et al., 2004).

To determine whether vAcgp64 and vAcgp64−/HaF utilize the same host receptor-binding sites on Sf21 cells, psoralen-inactivated BVs (Weightman & Banks, 1999) were used as competitor for binding of infectious BVs. BVs were diluted to 1.0 × 10⁷ tissue culture infectious dose 50 (TCID₅₀) units ml⁻¹ in Grace’s insect medium (Invitrogen) containing 10 % FBS with a final concentration of 0.5 mg ml⁻¹ 4-aminomethyl-4.5.8-trimethyl-psoralen (Sigma) and exposed for 30 min to UV light (300 nm). The effect of the psoralen inactivation was confirmed by a TCID₅₀ assay (O’Reilly et al., 1992) showing no residual infectivity after treatment.

Twenty-four-well plates were seeded with 3.0 × 10⁵ Sf21 cells per well in 500 µl Grace’s insect medium containing 10 % FBS. After overnight incubation at 27 °C the plates were cooled down to 4 °C. Cells were incubated with 0, 1, 10 or 100 TCID₅₀ units per cell of inactivated vAcgp64 or Acgp64−/HaF, respectively, for 1 h at 4 °C. Subsequently, 1.0 TCID₅₀ units per cell of infectious virus was added, followed by 1.5 h incubation at 4 °C. Finally, the cells were washed three times in Grace’s insect medium containing 10 % FBS and incubated 24 h at 27 °C. Infected cells were stained for GUS activity according to the Bac-to-Bac manual (Invitrogen). The number of infected cells in each well of two independent experiments (each performed in triplicate) was counted and represented as percentage of infected cells relative to that of the infection without inactivated virus (0 TCID₅₀ units per cell, 100 % infection) (Fig. 2a).

Psoralen-inactivated vAcgp64 and Acgp64−/HaF reduced the number of cells infected with the homologous virus at a higher m.o.i. At an m.o.i. of 100 TCID₅₀ units per cell, the number of infected cells was reduced by 66–85 %. Thus, viruses with the same envelope fusion protein were able to compete for cellular binding sites. In contrast, inactivated vAcgp64−/HaF even at the highest m.o.i. could not prevent the infection of Acgp64−/SeF (Fig. 2a). This indicates that the different receptor usage is directly related to difference in type of envelope fusion protein.

F proteins of group II NPVs are more diverged than GP64 proteins of group I NPVs (<29 % and ≥50 % amino acids identical, respectively). Therefore, it might be possible that members of the group II NPVs use different receptors. To test this possibility a similar competition assay was used as in Fig. 2(a), but now with vAcgp64−/HaF and vAcgp64−/SeF.

---

**Fig. 1.** Schematic presentation of the pseudotyped gp64-null AcMNPV bacmids. (a) Cassettes containing an AcMNPV p6.9 promoter–GUS reporter and an envelope fusion protein gene (EFP) under the control of the AcMNPV gp64 promoter or (b) the CMV-ie1 promoter–GFP reporter and an envelope fusion protein gene (EFP) under the control of the AcMNPV gp64 promoter are inserted into the attB sites (indicated by right and left insertion sites, Tn7R and Tn7L) in the polyhedrin (polh) locus by Tn7-based transposition of a gp64-null AcMNPV bacmid (bMON14272) to generate (a) vAcgp64−/Acgp64, vAcgp64−/HaF and vAcgp64−/SeF or (b) vAcgp64−/Acgp64-CMVeGfp and vAcgp64−/SeF–CMVeGfp. A chloramphenicol acetyl transferase gene (cat) has been substituted for the gp64 gene (position 108 039–109 761 in the AcMNPV genome, Ayres et al., 1994) in this bacmid.
the latter containing the SeMNPV F protein (Figs. 1, 2b). The HearNPV and SeMNPV F proteins are 34% identical and 54% similar in amino acid composition. Also this time, psoralen-inactivated vAc gp64–/HaF and Ac gp64–/SeF reduced the number of cells infected with the homologous virus at higher m.o.i. However, inactivated vAc gp64–/HaF also reduced the number of Ac gp64–/HaF-infected cells. At an m.o.i. of 100 TCID50 units per cell the number of infected cells was reduced by more than 80%. These results indicate that at least the HearNPV and SeMNPV F proteins bind to the same receptor binding site of S21 cells.

Recently, AcMNPV was exploited as a gene therapy vector (reviewed by Hu, 2006). Various mammalian cells seem to contain a receptor for AcMNPV GP64 since AcMNPV is able to transduce several mammalian cell types (Kost & Condreay, 2002; Hu, 2006). The baculovirus F protein has more similarities to other mammalian viral fusion proteins, in particular to paramyxovirus F proteins, than GP64. For instance, the SeMNPV F protein is 12% identical and 38% similar to that of the human respiratory syncytial virus (HRSV). Furthermore, computer prediction by Misseri et al. (2003) showed that the three-dimensional structures of F protein homologues of group II NPVs, GVs and errantiviruses show significant similarities to the X-ray-determined structure of the Newcastle disease virus (NDV) F protein (Chen et al., 2001). Therefore, it is possible that mammalian cells also contain a baculovirus F protein receptor, which would extend the array of baculoviruses for gene therapy applications. However, for the baculovirus HearNPV it has already been shown that this virus is unable to transduce several mammalian cell types (Liang et al., 2005). To extend this study and to rule out that other HearNPV BV proteins were responsible for the transduction inability, two near-isogenic recombinant AcMNPV viruses vAc gp64–/Ac gp64–CMVgfp and vAc gp64–/SeF–CMVgfp were constructed (Fig. 1b). These viruses have been made by Tn7 transposition of an expression cassette, containing the cytomegalovirus (CMV) ie-1 promoter–GFP reporter (Van Loo et al., 2001) and the AcMNPV gp64 gene or the SeMNPV f gene under the control of the AcMNPV gp64 promoter, in the polyhedrin locus of a gp64-null AcMNPV bacmid (Fig. 1b). These bacmids were transfected into S21 cells in order to generate infectious BVs which were then used to transduce LLC-PK1 (Hull et al., 1976), BHK-21 (Macpherson & Stoker, 1962) and H35 (Balinska et al., 1982) cells, respectively. Twenty-four-well plates were seeded with 1.0 × 105 LLC-PK1 or H35 cells in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS or BHK-21 cells in Glasgow minimal essential medium supplemented with tryptose phosphate broth and 10% FBS and incubated for 24 h at 37 °C. Cells were incubated for 2 h with 200 μl medium containing 1, 10 or 100 TCID50 units per cell of vAc gp64–/Ac gp64–CMVgfp or vAc gp64–/SeF–CMVgfp for 2 h and washed twice. After 48 h cells were examined for GFP expression by UV microscopy. The recombinant virus vAc gp64–/Ac gp64–CMVgfp was able to transduce all three cell types (Table 1). LLC-PK1 and BHK-21 cells containing GFP could be observed when 10 TCID50 units per cell were used, while GFP expression in H35 cells was found only at 100 TCID50 units per cell. However, vAc gp64–/SeF–CMVgfp was not able to transduce any of the mammalian cell types at the maximal attainable m.o.i. of 100 TCID50 units per cell. The finding that inability to
enter mammalian cells is only due to the F protein together with the results of Liang et al. (2005) strongly suggests that mammalian cells do not possess a receptor for baculovirus F proteins, despite the high degree of structural homology with envelope fusion proteins of mammalian viruses.

Thus, baculovirus envelope fusion proteins F and GP64 recognize distinct receptors to gain entry into cultured insect cells. The nature of these receptors is still enigmatic. For infections including heparan sulfate for efficient gene transfer in mammalian cells. The genome sequence and evolution of baculoviruses. The complete DNA sequence of Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrane fusion. Structure 9, 255–266.

Table 1. Mammalian cell transduction ability of two pseudo-typed gp64-null AcMNPV viruses at different m.o.i.

<table>
<thead>
<tr>
<th>TCID&lt;sub&gt;50&lt;/sub&gt; units per cell</th>
<th>vAc&lt;sup&gt;gp64&lt;/sup&gt;-/Acgp64</th>
<th>vAc&lt;sup&gt;gp64&lt;/sup&gt;-/SeF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1 BHK-21 H35</td>
<td>LLC-PK1 BHK-21 H35</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Acknowledgements

We thank Dr D. Zuidema (Wageningen University, The Netherlands) for his advice during the research, Dr B. J Scholte (Department of Cell Biology and Genetics, Erasmus Medical Center, The Netherlands) for kindly providing the CMV ie-1 promoter-GFP reporter plasmid and the CCL-PK1 cells and Dr T. K. F Schulz (Department of Molecular Cell Biology, Institute of Biomembranes, Utrecht University, The Netherlands) for kindly providing the H35 cells. This research was supported in part by a grant from the Royal Netherlands Academy of Arts and Sciences (KNAW) (Program Strategic Scientific Alliances project 04-PSA-BD-02).

References


