Journal of General Virology (2007), **88**, 3302–3306 **DOI** 10.1099/vir.0.83240-0

Short Communication

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

Correspondence
Just M. Vlak
just.vlak@wur.nl

Received 14 June 2007
Accepted 30 July 2007

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. *Sf*21 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.

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; Hayakawa *et al.*, 2000; Herniou *et al.*, 2001, 2003). The budded virus (BV) phenotype of group I NPVs [e.g. *Autographa californica* multiple NPV (AcMNPV)] contains a GP64-like major envelope glycoprotein. This protein is involved in viral attachment to host insect cells (Hefferon *et al.*, 1999), triggers low-pH-dependent membrane fusion during BV entry by endocytosis (Blissard & Wenz, 1992; Kingsley *et al.* 1999; Plonsky *et al.*, 1999; Volkman & Goldsmith., 1985) and is required for efficient budding of BVs from the cell surface (Monsma *et al.*, 1996; Oomens & Blissard 1999). BVs of group II NPVs and GVs lack a homologue of GP64. The low-pH-dependent membrane fusion during BV entry by endocytosis is triggered in this case by the major envelope glycoprotein F (IJkel *et al.*, 2000; Pearson *et al.*, 2000). GP64 in AcMNPV BVs can be replaced by the F protein of group II NPVs (Long *et al.*, 2006; Lung *et al.*, 2002), indicating that F is functionally analogous to GP64.

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, vAc$^{gp64-/Acgp64}$

†Present address: Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands.

and vAc$^{gp64-/HaF}$, 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 Tn*7* 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 *Sf*21 cells (Vaughn *et al.*, 1977) in order to generate infectious BVs as described previously (Westenberg *et al.*, 2004).

To determine whether vAc$^{gp64-/Acgp64}$ and vAc$^{gp64-/HaF}$ utilize the same host receptor-binding sites on *Sf*21 cells, psoralen-inactivated BVs (Weightman & Banks, 1999) were used as competitor for binding of infectious BVs. BVs were diluted to 1.0×10^7 tissue culture infectious dose 50 ($TCID_{50}$) 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-trimethylpsoralen (Sigma) and exposed for 30 min to UV light (300 nm). The effect of the psoralen inactivation was confirmed by a $TCID_{50}$ assay (O'Reilly *et al.*, 1992) showing no residual infectivity after treatment.

Twenty-four-well plates were seeded with 3.0×10^5 *Sf*21 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 $TCID_{50}$ units per cell of inactivated vAc$^{gp64-/Acgp64}$ or Ac$^{gp64-/HaF}$, respectively, for 1 h at 4 °C. Subsequently, 1.0 $TCID_{50}$ 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 $TCID_{50}$ units per cell, 100 % infection) (Fig. 2a).

Psoralen-inactivated vAc$^{gp64-/Acgp64}$ and Ac$^{gp64-/HaF}$ reduced the number of cells infected with the homologous virus at a higher m.o.i. At an m.o.i. of 100 $TCID_{50}$ 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 vAc$^{gp64-/Acgp64}$ even at the highest m.o.i. could not prevent the infection of Ac$^{gp64-/HaF}$ (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 ($\geqslant$29 % and $\geqslant$50 % amino acids identical, respectively). Therefore, it might be possible that members of the group II NPVs use different receptors. To test this possibility a similar competition assay was used as in Fig. 2(a), but now with vAc$^{gp64-/HaF}$ and vAc$^{gp64-/SeF}$,

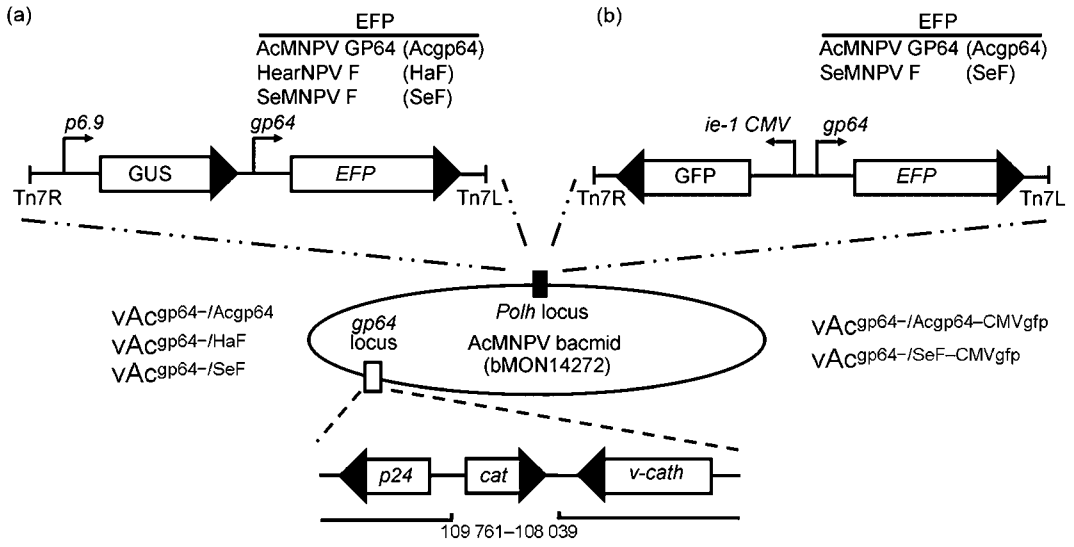

Fig. 1. Schematic presentation of the pseudotyped *gp64*-null AcMNPV bacmids. (a) Cassettes containing an AcMNPV *p6.9* promoter–GUS reporter and an envelope fusion protein gene (EFP) under the control of the AcMNPV *gp64* promoter or (b) the CMV-*ie1* promoter–GFP reporter and an envelope fusion protein gene (EFP) under the control of the AcMNPV *gp64* promoter are inserted into the *att b* sites (indicated by right and left insertion sites, Tn7R and Tn7L) in the polyhedrin (*polh*) locus by Tn*7*-based transposition of a *gp64*-null AcMNPV bacmid (bMON14272) to generate (a) vAc$^{gp64-/Acgp64}$, vAc$^{gp64-/HaF}$ and vAc$^{gp64-/SeF}$ or (b) vAc$^{gp64-/Acgp64-CMVgfp}$ and vAc$^{gp64-/SeF-CMVgfp}$. 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.

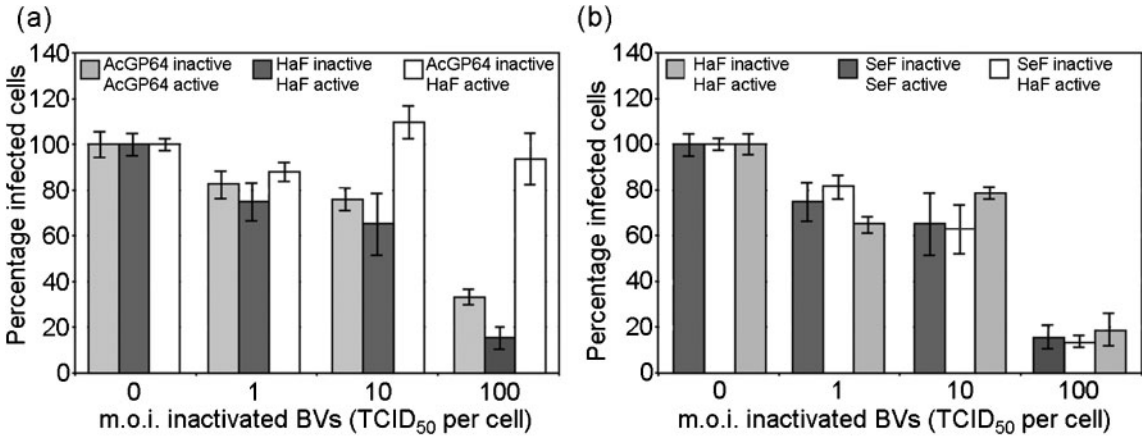

Fig. 2. Competition between *gp64*-null AcMNPV BVs pseudotyped with (a) AcMNPV GP64 or HearNPV F and (b) HearNPV F and SeMNPV F for host receptor binding sites on *Sf*21 cells. Psoralen-inactivated pseudotyped *gp64*-null AcMNPV BVs were used as competitors for binding of infectious pseudotyped *gp64*-null AcMNPV BVs. *Sf*21 cells were pre-incubated with increasing concentrations of psoralen-treated BVs (*x*-axis, m.o.i.) at 4 °C for 1 h, then infectious BVs (m.o.i.=1 $TCID_{50}$ unit per cell) were added and allowed to bind for 1.5 h at 4 °C. Cells were washed three times with medium and incubated for 24 h at 27 °C. Infected cells were stained for GUS activity and counted by light microscopy. The amount of infected cells is presented as percentage in relation to the control (m.o.i.=0 $TCDI_{50}$ unit per cell, 100 % infection). Each data point represents average of two independent assays with each containing triplicate infections. Bars represent standard error of the mean (SE).

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 $vAc^{gp64-/SeF}$ also reduced the number of $Ac^{gp64-/HaF}$-infected cells. At an m.o.i. of 100 $TCID_{50}$ 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 *Sf*21 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 $vAc^{gp64-/Acgp64–CMVgfp}$ and $vAc^{gp64-/SeF–CMVgfp}$ were constructed (Fig. 1b). These viruses have been made by Tn*7* 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 *Sf*21 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 1.0×10^5 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 $TCID_{50}$ units per cell of $vAc^{gp64-/Acgp64–CMVgfp}$ 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 $vAc^{gp64-/Acgp64–CMVgfp}$ was able to transduce all three cell types (Table 1). LLC-PK1 and BHK-21 cells containing GFP could be observed when 10 $TCID_{50}$ units per cell were used, while GFP expression in H35 cells was found only at 100 $TCID_{50}$ 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 $TCID_{50}$ units per cell. The finding that inability to

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

+, Transduced; −, not transduced.

$TCID_{50}$ units per cell	$vAc^{gp64-/Acgp64}$			$vAc^{gp64-/SeF}$		
	LLC-PK1	BHK-21	H35	LLC-PK1	BHK-21	H35
1	−	−	−	−	−	−
10	+	+	−	−	−	−
100	+	+	+	−	−	−

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 group I NPVs the insect-cell receptor for GP64 may be a non-glycosylated protein (Park *et al.*, 1999; Wang *et al.*, 1997; Wickham *et al.*, 1992). Electrostatic interactions seem to play a role as well (Wang *et al.* 1997), which is further corroborated by the observation that AcMNPV can be purified by cation-exchange chromatography (Barsoum, 1999). In the case of GP64, heparan sulfate glycosaminoglycans seem to play an important role in the entry of group I NPVs (e.g. AcMNPV) into mammalian cells (Duisit *et al.*, 1999). However, when BVs are treated with heparan sulfate or insect cells with heparinases or polybrene prior to infection, the infectivity of AcMNPV and SeMNPV remained unaffected (M.W., unpublished data), suggesting that heparan sulfate glycosaminoglycans are not involved in insect cell entry of either group I or II NPVs. Therefore, further studies are necessary to elucidate to which molecules baculoviruses attach on insect and mammalian cells.

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

Adams, J. R. & McClintock, J. T. (1991). Nuclear polyhedrosis viruses of insects. In: *Atlas of Invertebrate Viruses*. Edited by J. R. Adams & J. R. Bonami. Boca Raton, FL: CRC Press.

Ayres, M. D., Howard, S. C., Kuzio, J., Lopez-Ferber, M. & Possee, R. D. (1994). The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology* **202**, 586–605.

Balinska, M., Samsonoff, W. A. & Galivan, J. (1982). Reversibly permeable hepatoma cells in culture. *Biochim Biophys Acta* **721**, 253–261.

Barsoum, J. (1999). Concentration of recombinant baculovirus by cation-exchange chromatography. *Biotechniques* **26**, 834–840.

Blissard, G. W. & Wenz, J. R. (1992). Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. *J Virol* **66**, 6829–6835.

Bulach, D. M., Kumar, C. A., Zaia, A., Liang, B. & Tribe, D. E. (1999). Group II nucleopolyhedrovirus subgroups revealed by phylogenetic analysis of polyhedrin and DNA polymerase gene sequences. *J Invertebr Pathol* **73**, 59–73.

Chen, L., Gorman, J. J., McKimm-Breschkin, J., Lawrence, L. J., Tulloch, P. A., Smith, B. J., Colman, P. M. & Lawrence, M. C. (2001). 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.

Duisit, G., Saleun, S., Douthe, S., Barsoum, J., Chadeuf, G. & Moullier, P. (1999). Baculovirus vector requires electrostatic interactions including heparan sulfate for efficient gene transfer in mammalian cells. *J Gene Med* **1**, 93–102.

Hayakawa, T., Rohrmann, G. F. & Hashimoto, Y. (2000). Patterns of genome organization and content in lepidopteran baculoviruses. *Virology* **278**, 1–12.

Hefferon, K. L., Oomens, A. G., Monsma, S. A., Finnerty, C. M. & Blissard, G. W. (1999). Host cell receptor binding by baculovirus GP64 and kinetics of virion entry. *Virology* **258**, 455–468.

Herniou, E. A., Luque, T., Chen, X., Vlak, J. M., Winstanley, D., Cory, J. S. & O'Reilly, D. R. (2001). Use of whole genome sequence data to infer baculovirus phylogeny. *J Virol* **75**, 8117–8126.

Herniou, E. A., Olszewski, J. A., Cory, J. S. & O'Reilly, D. R. (2003). The genome sequence and evolution of baculoviruses. *Annu Rev Entomol* **48**, 211–234.

Hu, Y. C. (2006). Baculovirus vectors for gene therapy. *Adv Virus Res* **68**, 287–320.

Hull, R. N., Cherry, W. R. & Weaver, G. W. (1976). The origin and characteristics of a pig kidney cell strain, LLC-PK. *In Vitro* **12**, 670–677.

IJkel, W. F. J., Westenberg, M., Goldbach, R. W., Blissard, G. W., Vlak, J. M. & Zuidema, D. (2000). A novel baculovirus envelope fusion protein with a proprotein convertase cleavage site. *Virology* **275**, 30–41.

Kingsley, D. H., Behbahani, A., Rashtian, A., Blissard, G. W. & Zimmerberg, J. (1999). A discrete stage of baculovirus GP64-mediated membrane fusion. *Mol Biol Cell* **10**, 4191–4200.

Kost, T. A. & Condreay, J. P. (2002). Recombinant baculoviruses as mammalian cell gene-delivery vectors. *Trends Biotechnol* **20**, 173–180.

Kuzio, J., Pearson, M. N., Harwood, S. H., Funk, C. J., Evans, J. T., Slavicek, J. M. & Rohrmann, G. F. (1999). Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. *Virology* **253**, 17–34.

Liang, C., Song, J. & Chen, X. (2005). The GP64 protein of *Autographa californica* multiple nucleopolyhedrovirus rescues *Helicoverpa armigera* nucleopolyhedrovirus transduction in mammalian cells. *J Gen Virol* **86**, 1629–1635.

Long, G., Westenberg, M., Wang, H., Vlak, J. M. & Hu, Z. (2006). Function, oligomerization and *N*-linked glycosylation of the *Helicoverpa armigera* single nucleopolyhedrovirus envelope fusion protein. *J Gen Virol* **87**, 839–846.

Lung, O., Westenberg, M., Vlak, J. M., Zuidema, D. & Blissard, G. W. (2002). Pseudotyping *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV): F proteins from group II NPVs are functionally analogous to AcMNPV GP64. *J Virol* **76**, 5729–5736.

Macpherson, I. & Stoker, M. (1962). Polyoma transformation of hamster cell clones–an investigation of genetic factors affecting cell competence. *Virology* **16**, 147–151.

Misseri, Y., Labesse, G., Bucheton, A. & Terzian, C. (2003). Comparative sequence analysis and predictions for the envelope glycoproteins of insect endogenous retroviruses. *Trends Microbiol* **11**, 253–256.

Monsma, S. A., Oomens, A. G. & Blissard, G. W. (1996). The GP64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. *J Virol* **70**, 4607–4616.

O'Reilly, D. R., Miller, L. K. & Luckow, V. A. (1992). *Baculovirus Expression Vectors: a Laboratory Manual*. New York: W. H. Freeman and Co.

Onstad, D. W. (2007). EDWIP: Ecological Database of the World's Insect Pathogens. Illinois Natural History Survey, Champaign, Illinois [accessed in June 2007, last update: December 2002]. http://cricket.inhs.uiuc.edu/edwipweb/edwipabout.htm

Oomens, A. G. & Blissard, G. W. (1999). Requirement for GP64 to drive efficient budding of *Autographa californica* multicapsid nucleopolyhedrovirus. *Virology* **254**, 297–314.

Park, J. O., Chang, K. H., Lee, H. H. & Chung, I. S. (1999). Biochemical analysis of *Hyphantria cunea* NPV attachment to *Spodoptera frugiperda* 21 cells. *Cytotechnology* **31**, 159–163.

Pearson, M. N., Groten, C. & Rohrmann, G. F. (2000). Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the *Baculoviridae*. *J Virol* **74**, 6126–6131.

Plonsky, I., Cho, M. S., Oomens, A. G., Blissard, G. & Zimmerberg, J. (1999). An analysis of the role of the target membrane on the Gp64-induced fusion pore. *Virology* **253**, 65–76.

Van Loo, N. D., Fortunati, E., Ehlert, E., Rabelink, M., Grosveld, F. & Scholte, B. J. (2001). Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. *J Virol* **75**, 961–970.

Vaughn, J. L., Goodwin, R. H., Tompkins, G. J. & McCawley, P. (1977). The establishment of two cell lines from the insect *Spodoptera frugiperda* (*Lepidoptera*; *Noctuidae*). *In Vitro* **13**, 213–217.

Volkman, L. E. & Goldsmith, P. A. (1985). Mechanism of neutralization of budded *Autographa californica* nuclear polyhedrosis virus by a monoclonal antibody: inhibition of entry by adsorptive endocytosis. *Virology* **143**, 185–195.

Wang, P., Hammer, D. A. & Granados, R. R. (1997). Binding and fusion of *Autographa californica* nucleopolyhedrovirus to cultured insect cells. *J Gen Virol* **78**, 3081–3089.

Weightman, S. A. & Banks, M. (1999). Photochemical inactivation of recombinant baculovirus. *J Virol Methods* **81**, 179–182.

Westenberg, M., Veenman, F., Roode, E. C., Goldbach, R. W., Vlak, J. M. & Zuidema, D. (2004). Functional analysis of the putative fusion domain of the baculovirus envelope fusion protein F. *J Virol* **78**, 6946–6954.

Wickham, T. J., Shuler, M. L., Hammer, D. A., Granados, R. R. & Wood, H. A. (1992). Equilibrium and kinetic analysis of *Autographa californica* nuclear polyhedrosis virus attachment to different insect cell lines. *J Gen Virol* **73**, 3185–3194.

Yanase, T., Yasunaga, C. & Kawarabata, T. (1998). Replication of *Spodoptera exigua* nucleopolyhedrovirus in permissive and non-permissive lepidopteran cell lines. *Acta Virol* **42**, 293–298.