In vitro host range of Autographa californica nucleopolyhedrovirus recombinants lacking functional p35, iap1 or iap2

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We have examined the host range in different insect cell lines of Autographa californica nucleopolyhedrovirus (AcMNPV) recombinants lacking p35, iap1 or iap2. These genes encode, or are predicted to encode, anti-apoptotic proteins. Abrogation of p35 reduced the ability of AcMNPV to replicate in permissive cell lines derived from Spodoptera frugiperda insects by inducing apoptosis. In semi-permissive cell lines, such as Lymantria dispar and Spodoptera littoralis cells, we observed cytopathic effects after infection with AcMNPV but little virus production. Infection of these cells by AcMNPV lacking p35 resulted in apoptosis. However, p35-deficient viruses were still able to replicate normally in Trichoplusia ni, Mamestra brassicae and Panolis flammea cell lines. Disruption of AcMNPV iap1 and iap2 was found not to affect virus replication in any of the cell lines. It was also possible to disrupt both iap1 and iap2 in the same virus without loss of infectivity. A virus without iap1 and p35 demonstrated identical growth characteristics and host range to a virus lacking p35. We conclude that in cells which respond to AcMNPV infection by initiating programmed cell death, the p35 gene product alone is sufficient to inhibit apoptosis. Removal of iap1 or iap2 has no effect on virus replication, even in cell lines which do not undergo apoptosis in response to AcMNPV infection. Our results with two semi-permissive cell lines further indicate that whilst p35 is important in blocking block apoptosis, other factors are involved in restricting AcMNPV replication within these cells.

Introduction

Autographa californica nucleopolyhedrovirus (AcMNPV) p35 can prevent apoptosis in virus-infected Spodoptera frugiperda cells (Clem et al., 1991). Viruses lacking a functional p35 induce plasma-membrane blebbing, nuclear condensation, internucleosomal cleavage of cellular DNA and yield less infectious progeny than unmodified controls. p35, however, is not required for virus replication in AcMNPV-infected Trichoplusia ni cells in vitro and in vivo. Homologues of p35 have so far been located only in AcMNPV and its closely related variant Bombyx mori (Bm)NPV (Kamita et al., 1993) where, although it is required to inhibit apoptosis, its absence does not affect virus replication. AcMNPV P35 comprises 299 amino acids with a molecular mass of 34.8 kDa. It has a high lysine content (10.7%) but lacks any distinctive sequence motifs. P35 can also block apoptosis in Drosophila melanogaster (Hay et al., 1995) and Caenorhabditis elegans (Sugimoto et al., 1994).

P35 may not be the sole inhibitor of apoptosis in baculovirus-infected insect cells. In AcMNPV-infected Spodoptera littoralis cells, P35 does not appear to be sufficient to block apoptosis (Chejanovsky & Gershburg, 1995). Further, p35 has not been detected in the complete sequence of Orgyia pseudotsugata (Op)MNPV (Ahrens et al., 1997). Instead, OpMNPV has another inhibitor of apoptosis gene (iap), the product of which probably blocks the same process in cells infected with this virus (Birnbaum et al., 1994). This conclusion, however, derives from the finding that OpMNPV iap (Op-iap) can complement AcMNPV mutants deficient in p35. P35 and
IAP probably act at different points in the apoptotic pathway (Clem et al., 1996). Op-iap has yet to be directly manipulated in the OpMNVP genome to determine its role in virus-infected cells. An iap was first identified in Cydia pomonella granulovirus (CpGV) via a similar ability to complement AcMNVP p35 mutants (Crook et al., 1993). In the absence of the complete sequence of CpGV, it is not known whether or not this virus has a copy of p35. Cp-iap and Op-iap constitute members of a family of apoptosis inhibitors which are unrelated to p35.

The AcMNVP genome also contains two iap-like sequences, designated iap1 and iap2 (Braunagel et al., 1993; Crook et al., 1993; Ayres et al., 1994). The Cp-iap, Op-iap and Ac-iap1 predicted protein sequences contain no recognizable signal sequences, transmembrane domains or nuclear localization signals. Baculovirus IAPs all contain carboxy-terminal Cys2-His-Cys4 (C3HC4) zinc- (or RING)-finger-like motifs, found in a number of transcriptional regulatory proteins (Freemont et al., 1991). Additional cysteine/histidine motifs which form repeat regions termed baculovirus IAP repeats (BIRs) are present in the N-terminal regions of the IAP polypeptides (reviewed in Clem et al., 1996). The conservation of iap sequences in baculoviruses, Drosophila (Hay et al., 1995; Duckett et al., 1996) and humans (Roth et al., 1995; Roy et al., 1995; Liston et al., 1996) suggests that they are involved in the regulation of apoptosis in various cellular systems. AcMNVP iap2 lacks coding regions for the distinctive BIRs but does retain the carboxy-terminal zinc-finger motifs.

Three further iap-like sequences have been identified in the OpMNVP genome (Ahrens et al., 1997). Two of these, Op-iap1 and Op-iap2, contain complete open reading frames and have the potential to encode proteins with 62 and 55% identity with the AcMNVP homologues respectively. A third iap-like gene (Op-iap3) corresponds to the original Op-iap described above and has been renamed to maintain consistency with the AcMNVP example. A fourth sequence, Op-iap4, has the potential to encode a truncated protein.

The putative products of AcMNVP iaps appear to be non-functional as inhibitors of apoptosis, since p35 mutants induce programmed cell death in virus-infected S. frugiperda cells. The AcMNVP IAPs, unlike P35 or Cp-IAP or Op-IAP, are unable to protect cells against actinomycin D-induced apoptosis (Clem et al., 1991; Clem & Miller, 1994). It was proposed that Ac-iap1 might be required to prevent apoptosis in cells from a different tissue of a particular insect host or could have lost the ability to prevent cell death since AcMNVP acquired p35 (Clem et al., 1996). The presence of iap-1 and iap-3 in OpMNVP suggests that iap-1 may never have had a role in preventing apoptosis since iap-3 probably fulfills this function for this virus. Gene duplication of an ancestral iap-like sequence or repeated acquisition of such sequences from another source may account for the multiple copies of these genes.

In this study, we have examined the requirements for AcMNVP p35, iap1 and iap2 in a number of insect cell lines by producing recombinant viruses lacking each of these genes. Viruses deficient in both p35 and iap1 or iap1 and iap2 were also constructed and tested in vitro to determine if multiple gene deletions might reveal a function for these sequences.

**Methods**

**Cells and viruses.** Spodoptera frugiperda IPLB-SF-21-AE (Sf21; Vaughn et al., 1977), Manduca sexta baculovirus (King et al., 1991), Trichoplusia ni (TN-368; Hink, 1970), Panolis flammea and Spodoptera littoralis cells (the latter two derived from neonate larval tissue; R. D. Possee, unpublished) were routinely maintained in TC100 media [Gibco, or prepared from basic ingredients as described by King & Possee (1992)] supplemented with 10% foetal calf serum (FCS). Lymantria dispar (Ld652Y) cells (Goodwin et al., 1978) were grown in S900 II media (Gibco) supplemented with 5% FCS. The AcMNVP used in these studies was the C6 strain (Possee, 1986), along with BacPAK6 virus (Kitts & Possee, 1993).

**Plasmid transfer vectors**

pAciap1.1. The SacI DNA fragment spanning the AcMNVP genome between nucleotides 21828 to 23559 (Ayres et al., 1994) was inserted into pUC118 and digested with Hpal to remove the portion between bp 22756 and 23017. The vector was recircularized by inserting an oligonucleotide adaptor (5’- CAGATCTG - 3’) to alter the restriction site to BglII (underlined). The resulting plasmid, pAciap1.1, contained the 52 amino-terminal codons of iap1, followed by two extra codons and a stop codon, introduced by the linker sequence. The Escherichia coli β-galactosidase (lacZ) coding region was ligated into the BglII site of this plasmid before the stop codon described above. The resulting plasmid, pAciap1.2, contained the amino-terminal portion (52 codons) of the iap1 gene fused to the lacZ coding region.

pAciap2.2. The SacI-G region of the AcMNVP genome (bp 60448–63194) was inserted into pUC118 and digested with Clal and SacII to remove the portion between bp 61263 and 61560. The cut ends of the vector were repaired with Klenow, converted to a BgII site, and the lacZ coding region ligated into the BgIII linker, as described above, to produce pAciap2.2. This plasmid contained a truncated iap2 sequence with the lacZ coding region fused with the 83 amino-terminal iap2 codons.

pAcp35.Z. The EcoRI-S region of the AcMNVP genome (bp 116040–117492) was inserted into pUC7 (pEcoRI-S), subsequently digested with HindIII (bp 116884) and ligated with a BgII oligonucleotide adapter (5’- AGCTAGATCTG - 3’) underlined). The lacZ coding region, under control of the AcMNVP polyhedrin promoter, was ligated as a 3.9 kb BamHI–BgII cassette from plasmid pAcR23 + lacZ (Possee & Howard, 1987) into the BgII site, generating pAcp35.Z.

pAcp35A. The EcoRI-S fragment, within pUC7, was digested with BstXI and HindIII to remove the region between bp 116572 and 116883, subsequently repaired with Klenow and recircularized to derive pAcp35A.

**Generation of recombinant baculoviruses.** Sf21 or TN-368 cells were co-transfected with viral DNA and transfer plasmids using standard protocols (King & Possee, 1992). Recombinant viruses Aciap1.Z, Aciap2.Z and Acp35.Z were generated from co-transfection with AcMNVP C6 virus DNA and plasmids pAciap1.Z, pAciap2.Z and pAcp35.Z, respectively. Aciap1.Z was produced following co-transfection of Aciap1.Z virus DNA with plasmid pAciap1Δ; subsequent co-transfection of Aciap1Δ viral DNA with pAciap2.Z derived the virus double
mutant Aciap1<i>Δiap2</i>2. A<i>cp35Δ</i> was generated after co-transfection of A<i>cp35</i> virus DNA with plasmid pA<i>cp35Δ</i>; subsequent co-transfection of A<i>cp35</i> viral DNA with pA<i>ciap1</i>2 produced virus A<i>cp35</i>iap1<i>Δiap2</i>1. Viruses lacking a functional p<i>35</i> coding region were generated, selected and propagated in TN-368 cells; all other viruses were grown in SF21 cells. Recombinant viruses were screened using the presence or absence of lacZ as a genetic marker in plaque-purification assays incorporating X-Gal chromogenic substrate (King & Possee, 1992).

- **Verification of virus-induced apoptosis in insect cells.** SF21 or TN-368 cells (5 x 10<sup>5</sup>) were infected at an m.o.i. of 10 p.f.u. per cell and incubated at 28 °C for up to 3 days post-infection (p.i.). Cells were examined under the light microscope for the occurrence of apoptosis (≥10 h p.i.), characterized by severe cell blebbing, or evidence of occluded virus production. For rescue of apoptotic phenotype, SF21 cells were co-transfected with 0.5 µg viral DNA and 2.5 µg pEcoRI-S DNA, and incubated at 28 °C for 3 days.

- **Virus growth and titration analyses.** SF21 cells seeded into 35 mm dishes at 5 x 10<sup>5</sup> cells per dish were inoculated with viruses at an m.o.i. of 10 p.f.u. per cell, and incubated at 28 °C for 1 h. Monolayers were washed three times with TC100–FCS and further incubated at 28 °C. Culture supernatants were harvested and clarified at various times p.i., and infectious virus titres determined by endpoint dilution analysis in plaque assays (King & Possee, 1992).

- **In vitro host range studies.** Insect cell lines (5 x 10<sup>5</sup> cells per 35 mm dish) were infected at an m.o.i. of 20 p.f.u. per cell with wild-type or recombinant virus and incubated for 10 to 48 h at 28 °C. Cells were examined under the light microscope for evidence of occluded virus production or the occurrence of apoptosis. Infected cell culture medium was harvested at 48 h p.i. and titrated by plaque assay using TN-368 cells. Virus yields were compared using Student’s t-test statistical analysis.

## Results

**Construction of recombinant viruses with incomplete iap<i>1</i> or iap<i>2</i>**

Recombinant AcMNPVs with modified iap<i>1</i> and iap<i>2</i> were produced by inserting lacZ into the coding region of each gene to derive A<i>ciap1</i>1 and A<i>ciap2</i>2, respectively. The genetic organization of each recombinant virus is illustrated in Fig. 1 and the respective parent listed in Table 1. The lacZ sequences in A<i>ciap1</i>1 replaced nucleotides 22756–23017 of the virus genome corresponding to 157–418 bp of the iap<i>1</i> coding region (ATG = +1). In A<i>ciap2</i>2, lacZ replaced nucleotides 61263–61500, equivalent to 247–543 bp of iap<i>2</i>. The recombinant virus genomes were examined by digesting with restriction enzymes and hybridization with radiolabelled probes to confirm the genetic alteration (data not shown). The remainder of the virus genomes was unchanged from the original parental stock used in the co-transfection.

Working stocks of wild-type and recombinant viruses were prepared and titrated using SF21 cells. Although the recombinant viruses showed a slight reduction in infectivity when compared with the AcMNPV wild-type (Table 1), the titres for A<i>ciap1</i>1 and A<i>ciap2</i>2 were comparable with BacPAK6 (Kitts & Possee, 1993), which also contains the lacZ coding region.

Virus replication was examined more closely in a one-step growth curve by infecting SF21 cells with 10 p.f.u. per cell and monitoring infectious virus production between 0 and 48 h p.i. (Fig. 2). There were no apparent differences in the time of appearance of infectious budded virus in the medium of cultures infected with A<i>ciap1</i>1Z (Fig. 2a), A<i>ciap2</i>2Z (Fig. 2b) or BacPAK6. We also monitored the virus-infected SF21 cells throughout the period of this experiment for plasma-membrane blebbing, which is indicative of apoptosis in insect cells (Clem et al., 1991). Cells infected with AcMNPV, A<i>ciap1</i>1Z or A<i>ciap2</i>2Z showed no signs of programmed cell death (Fig. 3b, c, d respectively) nor did cells infected with BacPAK6 (data not shown). Conversely, SF21 cells infected with an AcMNPV recombinant containing lacZ inserted within p35 (A<i>cp35</i>Z, see Fig. 1) had the characteristic features of apoptosis (Fig. 3f).

**Construction of a recombinant virus lacking both iap<i>1</i> and iap<i>2</i>**

The A<i>ciap1</i>1Z recombinant virus was further modified to remove the lacZ sequences and delete part of the iap<i>1</i> coding sequence. Genomic DNA from A<i>ciap1</i>1Z was mixed with plasmid pA<i>ciap1</i>Δ and used to co-transfect SF1 cells. The recombinant virus generated, A<i>ciap1</i>1Δ, lacked nucleotides 22756–23017 which correspond to 157–418 bp of the coding region of iap<i>1</i> (Fig. 1). A<i>ciap1</i>1Δ was then used as the parental virus to derive another recombinant deficient in both iap<i>1</i> and iap<i>2</i> (Fig. 2). Virus genomic DNA (A<i>ciap1</i>1Δ) was mixed with the plasmid (pA<i>ciap2</i>2Z) originally employed to produce A<i>ciap2</i>2Z, and used to co-transfect SF1 cells. After plaque purification of these virus recombinants (A<i>ciap1</i>1Δ and A<i>ciap1</i>1<i>Δiap2</i>2) and amplification of working stocks, both appeared to replicate normally in SF21 cells (Table 1). The genetic organization of the viruses was confirmed as before. A one-step growth curve showed that SF21 cells infected with 10 p.f.u. per cell of BacPAK6, A<i>ciap1</i>1Δ or A<i>ciap1</i>1<i>Δiap2</i>2Z produced infectious budded virus in a similar manner (Fig. 2). Cells infected with A<i>ciap1</i>1<i>Δiap2</i>2Z showed no signs of apoptosis (Fig. 3e) and produced polyhedra.

**Construction of a recombinant virus with non-functional p35 and incomplete iap<i>1</i>**

A recombinant virus with a modified p35 was produced by inserting a polyhedrin gene promoter-lacZ cassette into the coding region to derive A<i>cp35</i>Z (Fig. 1). The genetic organization of the virus was confirmed as before (data not shown). The lacZ coding region was then removed from this virus by co-transfecting TN-368 cells with A<i>cp35</i>Z DNA and pA<i>cp35Δ</i>. Recombination between genomic DNA of A<i>cp35</i>Z and pA<i>cp35Δ</i> should have replaced the lacZ-interrupted p35 with a truncated p35 coding sequence lacking 311 nucleotides between positions 116572 and 116883 of the complete AcMNPV genome (Ayres et al., 1994). After digesting A<i>cp35Δ</i>
Fig. 1. Linear representation of AcMNPV CG (top) and recombinant virus genomes, depicting manipulations of iap1, iap2 and p35 gene regions. AcMNPV virus sequences are represented by narrow lines or open boxes, E. coli lacZ coding sequence by thick black lines, and deleted regions by spliced lines. Direction of transcription (arrows) and gene limits (Ayres et al., 1994) are indicated.
Table 1. Propagation of recombinant viruses in insect cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>Parent virus*</th>
<th>Host cell†</th>
<th>Titre (p.f.u./ml)‡</th>
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<tr>
<td>AcMNPV C6</td>
<td>–</td>
<td>Sf21</td>
<td>6.3 × 10⁷</td>
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<tr>
<td>AcBacPAK6</td>
<td>AcMNPV</td>
<td>Sf21</td>
<td>3.9 × 10⁷</td>
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<tr>
<td>Aciap1.Z</td>
<td>AcMNPV</td>
<td>Sf21</td>
<td>4.0 × 10⁷</td>
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<td>Sf21</td>
<td>4.7 × 10⁷</td>
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<tr>
<td>Aciap1Δ</td>
<td>Aciap1.Z</td>
<td>Sf21</td>
<td>4.3 × 10⁷</td>
</tr>
<tr>
<td>Aciap1Δiap2.Z</td>
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<td>Sf21</td>
<td>4.8 × 10⁷</td>
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<tr>
<td>Acp35.Z</td>
<td>AcMNPV</td>
<td>TN-368</td>
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<td>TN-368</td>
<td>3.0 × 10⁶</td>
</tr>
<tr>
<td>Acp35Δiap1.Z</td>
<td>Acp35 Δ</td>
<td>TN-368</td>
<td>4.6 × 10⁶</td>
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</table>

* This virus was used to derive the corresponding recombinant virus in the first column.
† The insect cell line used to perform recombinant virus construction and subsequent amplification.
‡ Suspension cultures (50 ml; 5 × 10⁵ cells/ml) of Sf21 or TN-368 were infected with virus (0.1 p.f.u. per cell) and incubated for at 28 °C for 5 days. The culture medium was harvested and infectious virus titrated using the appropriate cell line.

Fig. 2. Growth curves for iap-interrupted viruses. Cells were infected (10 p.f.u. per cell) and samples harvested for titration at 4 h intervals from 0 to 48 h p.i. Virus titres (log p.f.u./ml) for Aciap1.Z (○), Aciap2.Z (●), Aciap1Δ (▲) and Aciap1Δiap2.Z (△) were plotted with respect to control virus BacPAK6 (○) against time p.i. Standard deviations were derived from three replicates.

DNA with EcoRI, however, the 1485 bp EcoRI S region was found to contain a 3 kbp insert which did not hybridize with AcMNPV DNA. Sequencing of this part of the Acp35Δ virus genome revealed the predicted recombination had not occurred, but that part of the lacZ coding region had been left intact (Fig. 1). In addition, a 14 bp insertion was detected, which comprised AT-rich DNA, at the p35–lacZ junction. Several other isolates of Acp35Δ were found to have the same aberrant genotype.

The phenotypes of Acp35.Z and Acp35Δ were examined in Sf21 and TN-368 cells. Apoptosis was observed only in Sf21 cells infected with either virus (data not shown). Co-trans-
Fig. 3. Morphology of Sf21 cells after mock infection (a) or infection with AcMNPV C6 (b), Aciap1.Z (c), Aciap2.Z (d), Aciap1Δiap2.Z (e) and Acp35.Z (f). Bar marker indicates 50 µm.
Cells were infected with Ac
mutants. At 48 h p.i., however, a cytopathic effect was evident, failed to produce polyhedra when infected with AcMNPV (Fig. 5d) or Ac35Δiap1.Z cells (Fig. 5e), with extensive cell blebbing.

The production of infectious budded virus was assessed in Sf21, TN-368, M. brassicae, Ld652Y and P. flammea cells (Fig. 6). The cells were infected with either BacPAK6, Ac35.Z, Aciap1.Z, Aciap2.Z or Ac35Δiap1.Z at an m.o.i. of 5 p.f.u. per cell. BacPAK6 was selected to represent wild-type virus, in place of AcMNPV C6, because it contained lacZ, as did the iap1/p35 mutants used in this study. Virus-infected cells were incubated for 48 h before harvesting the medium, which was titrated in TN-368 cells to assess virus yields (Fig. 6). The results showed that deletion of p35, iap1, iap2 or iap1 and p35 had little effect on infectious virus production in infected TN-368, P. flammea and M. brassicae cells, although there was some variation between cell lines. However, Sf21 cells infected with Ac35.Z or Ac35Δiap1.Z, as expected, produced 100-fold less infectious virus than when using BacPAK6. Infectious virus yields in Ld652Y cells were very low for all of the recombinant viruses used and probably comprise residual inoculum virus. The S. littoralis cells were not included in this study because preliminary studies had shown that challenging the cultures with AcMNPV failed to increase the titre of budded virus above levels which could be attributed to residual inoculum virus.

**Discussion**

This study has examined the importance of AcMNPV p35, iap1 and iap2 in determining virus host range in a number of insect cell lines. Baculoviral p35, which to date has only been

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<td></td>
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<td>Ap</td>
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<td>Mamestra brassicae</td>
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<td>Lymantria dispar 652Y</td>
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* Insect cells were inoculated with each virus at an m.o.i. of 20 p.f.u. per cell and incubated for 48 h at 28 °C.
† Productive virus infection of cell lines was determined by the appearance of virus occlusion bodies (OB).
‡ Abortive infections were characterized by apoptotic cell blebbing (Ap).
Fig. 4. Morphology of Ld652Y cells after mock infection (a) or infection with AcMNPV C6 (b), Aciap1.Z (c), Acp35.Z (d) and Acp35Δiap1.Z (e). Bar marker indicates 50 µm.
Fig. 5. Morphology of S. littoralis cells after mock infection (a) or infection with AcMNPV C6 (b), Aciap1.Z (c), Acp35.Z (d) and Acp35Δiap1.Z (e). Bar marker indicates 50 μm.
identified in AcMNPV and BmNPV (Clem et al., 1991; Kamita et al., 1993), produces a protein which inhibits apoptosis in virus-infected cells. An AcMNPV deficient in p35 has been demonstrated to have a reduced ability to replicate in Sf21 cells but replicates normally in TN-368 cells (Clem et al., 1991). We have extended these observations to show that a p35-deficient virus can also be propagated efficiently in M. brassicae and P. flammea cell lines to produce both budded and occluded virus progeny. However, in S. littoralis cells, which support very limited AcMNPV replication, viruses lacking functional p35 induced extensive apoptosis. Infection of S. littoralis SL2 cells with AcMNPV (E2) was previously documented to induce apoptosis, coinciding with low virus replication in these cells (Chejanovsky & Gershburg, 1995). In our hands, AcMNPV (C6) produced some apoptosis in S. littoralis cells and occluded virus was synthesized, albeit at lower levels than observed in Sf21 cells. Both the cell line and virus variant used in this study were different to those employed by Chejanovsky & Gershburg (1995). In other studies, AcMNPV has been observed to induce apoptosis in cell lines derived from the midgut of the spruce budworm, *Choristoneura fumiferana*, but replicates to produce occluded virus in ovarian cells from the same insects (Palli et al., 1996). Parallel studies with the SL2 cell line and the S. littoralis cells used in our experiments would be interesting to determine any differences in AcMNPV replication.

When Ld652Y cells are inoculated with AcMNPV, semi-permissive replication results in cytopathic effects, viral DNA replication and expression of viral mRNA, but no production of infectious virus (McClintock et al., 1986; Guzo et al., 1992). This block in AcMNPV replication can be overcome if Ld652Y cells are superinfected with *L. dispar* (Ld)MNPV (McClintock & Dougherty, 1987). The LdMNPV *hrf-1*, which promotes complete AcMNPV replication, has been identified (Thiem et al., 1996). The limited replication of AcMNPV in Ld652Y cells can be further inhibited if p35 is removed from the virus genome. We noted apoptosis in Ld652Y cells challenged with Acp35Z, Acp35Δ or Acp35Δiap1Z, indicating that P35 is required in this cell line. This result confirms observations made by Du & Thiem (1997). However, the requirement for *hrf-1* to overcome further blocks in AcMNPV replication in Ld652Y cells suggests that it would be wrong to attribute p35 sole responsibility for regulating the host range of this virus. It would be interesting to coinfec Ld652Y cells with Acp35Δ and LdMNPV to determine if the latter virus encodes an inhibitor of apoptosis.

We also assessed the role of *iap1* and *iap2* in AcMNPV infection of insect cells. The replication of AcMNPV in each of the lepidopteran cell lines tested was found to be unaffected by the disruption of *iap1* or *iap2* genes, resulting in virus yields comparable to those of control viruses. Furthermore, disruption of both *iap* genes in AcMNPV did not affect replication in Sf21 cells.
We also examined the growth characteristics of virus lacking p35 and iap1 in different cell lines, but no detectable differences were observed in the levels of virus progeny produced by viruses lacking both of these genes. In contrast to previous predictions (Clem & Miller, 1994b) that iap1 may be essential for infection of T. ni cell lines where viruses lack a functional p35 gene, we found that the virus Acp35Δiap1.Z was able to replicate normally in TN-368 cells. The successful production of a recombinant AcMNPV deficient in both p35 and iap1 by propagation in TN-368 cells suggests that iap1 is not required for the control of apoptosis in T. ni cells.

Our studies indicate that AcMNPV iaps have no significant influence on the in vitro host range of AcMNPV in the cell lines tested. Viruses which are deficient in both iap1 and p35 maintained the same host range as viruses deficient solely in p35, whilst viruses deficient in one or both iaps maintained the same host range as wild-type controls. These data suggest that the deletion of p35 is the main factor controlling replication ability of these viruses in the different host cells examined. The additional deletion of iap1 did not have any further effect on the ability of AcMNPV to maintain its host range. We conclude that iap1 (and probably iap2) has no apparent role in the host range determination of AcMNPV and does not prevent apoptosis in those cell lines which do not require p35 to inhibit apoptosis. It should be remarked, however, that the range of cell lines tested is by no means exhaustive, and may not include species in which IAP1 or IAP2 are required. Additionally, despite the ability of a virus deficient in both p35 and iap1 to replicate in TN-368 cells, our attempts to isolate a virus deficient in p35, iap1 and iap2 have proved unsuccessful. It is also not known at this time whether a mutant of AcMNPV deficient in p35 and iap2 is viable in T. ni cells.

It is likely that baculovirus IAP interacts to control apoptosis in insect cell lines, and this may occur in a cell-line-specific manner if the cellular targets of IAPs from different baculovirus species are themselves significantly different. The nucleotide sequence of OpMNPV-iap1 predicts 58% amino acid sequence identity to the product of CpGV-iap but only 28% amino acid sequence identity to AcMNPV-iap1. Despite this degree of identity between the iaps of AcMNPV and those of OpMNPV and CpGV, no function has yet been attributed to the AcMNPV iap products. Domain-swapping experiments with the active CpGV iap (Clem & Miller, 1994a) have failed to restore an anti-apoptotic function to AcMNPV iap1. It has been hypothesized that since acquiring the p35 anti-apoptotic gene, the AcMNPV iap genes have lost their function due to lack of selective pressure (Clem et al., 1996). In order to examine this, the cellular targets of baculovirus IAPs need to be fully defined. The cell interactions of baculovirus IAP are unknown, although the human IAPs are known to interact with the TNF receptor-associated factors (TRAFS) (Rothe et al., 1995). IAP may be the primary apoptosis-inhibiting protein encoded by some baculoviruses. However, AcMNPV and BmNPV have acquired p35, which may be active in conjunction with iap to inhibit apoptosis in a wider range of hosts (Clem & Miller, 1994b). This could explain the relatively broad host range of AcMNPV compared to other baculoviruses, in particular when compared to the closely related baculovirus OpMNPV, which lacks p35 (Gombart et al., 1989; Ahrens et al., 1997) and demonstrates a significantly narrower host range. We now plan to test the ability of the various virus recombinants described in this study to replicate in different species of insect larvae.

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References


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