Caspase inhibitor P35 is required for the production of robust baculovirus virions in *Trichoplusia ni* TN-368 cells

Bart Bryant and Rollie J. Clem

Molecular, Cellular and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506, USA

Apoptosis can protect lepidopteran insects against baculovirus infection by limiting viral replication. Baculoviruses counter this response by expressing anti-apoptotic proteins such as the caspase inhibitor P35, which is expressed by several baculoviruses including *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). Mutants of AcMNPV that lack the p35 gene induce apoptosis in *Spodoptera frugiperda* cells, and replication of these mutants is severely curtailed in *S. frugiperda* cell lines and larvae. However, cells from another lepidopteran species, *Trichoplusia ni*, do not undergo apoptosis when infected with AcMNPV mutants lacking p35, and p35 mutant and wild-type viruses replicate to equivalent levels in the *T. ni* cell line TN-368 and have equivalent infectivity in *T. ni* larvae by either oral or intrahaemocoelic injection. This has led to the conclusion that p35 is not required for AcMNPV replication in *T. ni*. However, in this study it was found that stocks of p35 mutant virus produced in TN-368 cells had defects in virion stability and infectivity. TN-368 cells infected with p35 mutant AcMNPV exhibited caspase activity, despite a lack of apoptosis, and propagation of the mutant virus in the presence of a chemical caspase inhibitor restored the normal infection phenotype to the progeny virus. These results suggest that caspases can directly or indirectly damage baculovirus virions, and reveal a novel aspect of the role of apoptosis in antiviral defence.

INTRODUCTION

The *Baculoviridae* are a family of large DNA viruses that are restricted to infection of arthropods, with most known baculoviruses infecting caterpillars, the larval stage of lepidopteran insects (for general reviews on baculoviruses, see Friesen, 2007; Okano *et al*., 2006). Similar to other types of large DNA viruses, baculoviruses encode many gene products that are involved in viral replication and in modulating host functions. For example, the genome of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), the best-studied baculovirus to date, is approximately 134 kb in length and is predicted to encode more than 150 genes (Ayres *et al*., 1994). During their replication cycle, baculoviruses produce two distinct types of virion, known as the budded (BV) and occlusion-derived (ODV) virus forms. Nucleocapsids, which are assembled in the nucleus, initially migrate to the cell periphery and bud through the plasma membrane, resulting in extracellular enveloped BVs. These BV particles are infectious in most cell types and are responsible for the spread of infection within the infected insect. Later in infection, ODVs are produced when the nucleocapsids are retained in the nucleus of the infected cell, acquire envelopes and become embedded in large protein crystals known as occlusion bodies. These ODV-containing occlusion bodies are highly stable in the environment and are responsible for horizontal spread of the virus to other susceptible hosts by oral infection. Caterpillars become infected by feeding on occlusion bodies, which dissolve in the caterpillar midgut and release ODVs that infect epithelial cells lining the midgut. The envelopes of BV and ODV differ in protein composition, such that ODVs are specialized for infection of midgut epithelial cells, whilst BVs can attach and enter almost any kind of cell, including mammalian cells.

The p35 gene has been shown to be a host-range-determining gene in AcMNPV due to its ability to inhibit apoptosis (Clem *et al*., 1991). AcMNPV mutants lacking p35 cause apoptosis during infection of *Spodoptera frugiperda* (the fall armyworm) cells and replicate poorly in cell lines from this species, such as the SF-21 cell line or its clonal derivative, S9. Such mutants also have defects in late gene expression in SF-21 cells (Clem & Miller, 1993; Hershberger *et al*., 1992). In addition, p35 mutants exhibit greatly reduced infectivity, time to death and occlusion body production in *S. frugiperda* larvae compared with wild-type virus, and these defects correlate well with apoptosis (Clarke & Clem, 2003; Clem & Miller, 1993; Clem *et al*., 1994). However, cells from *Trichoplusia ni* (the cabbage looper), such as TN-368 cells, are relatively...
resistant to a variety of apoptotic stimuli (Bonner & Liengswangwong, 1994; Clem et al., 1991; Koval & Kazmar, 1988), and mutant viruses lacking p35 do not cause apoptosis in TN-368 cells. Furthermore, production of BVs and occlusion bodies, as well as the overall timing of viral gene expression and DNA replication, has been reported to be similar to cells infected with wild-type AcMNPV (Clem & Miller, 1993; Hersherberger et al., 1992). Viruses lacking p35 also exhibit normal infectivity, time to death and occlusion body yields when infecting T. ni larvae by either the oral or intraheamocoeic routes. There is an observable difference, however, between the phenotypes of p35 mutant and wild-type AcMNPV in infected T. ni larvae; both T. ni and S. frugiperda larvae infected with p35 mutant viruses do not undergo liquefaction, a process of host degradation often seen during the final stages of baculovirus infection in lepidopteran larvae (Clem & Miller, 1993; Clem et al., 1994). This observation suggests that P35 plays some role in infection of T. ni.

During apoptosis in organisms ranging from nematodes to mammals, a family of cysteine proteases called caspases becomes activated by autolytic cleavage (reviewed by Riedl & Shi, 2004). Two main types of caspase exist, known as initiator and effector caspases. Following an apoptotic signal, initiator caspases become activated by autocatalytic cleavage. Activated initiator caspases cleave and activate effector caspases, which in turn cleave a number of cellular proteins, leading directly to apoptosis. AcMNPV infection causes caspase activation, but wild-type AcMNPV is able to inhibit caspase activity, and thereby apoptosis, by virtue of expressing P35, which is a highly effective inhibitor of many caspases, especially effector caspases (Clem, 2005). AcMNPV mutants that lack p35 cannot inhibit caspase activity in S. frugiperda cells, and high levels of caspase activity ensue, resulting in apoptosis of the infected cells. In contrast, cells derived from T. ni, such as TN-368 cells, do not undergo apoptosis following infection by p35 mutant AcMNPV.

Despite the fact that p35 mutant AcMNPV does not cause apoptosis in T. ni cells, we found that BV stocks of this mutant virus propagated in TN-368 cells had defects in virion stability and infectivity. The infection phenotype was rescued when virus was grown in the presence of a caspase inhibitor, suggesting that caspase activity can have detrimental effects on virions and affect the next round of virus replication. These results revealed a novel consequence of the insect apoptotic response to baculovirus infection.

**METHODS**

**Viruses and cells.** Construction of the viruses vHSGFP/P35del (referred to here as P35A) and vP35delRev (referred to here as Rev) has been described previously (Clarke & Clem, 2003). Both viruses express enhanced green fluorescent protein (eGFP) from a *Drosophila* hsp70 promoter inserted near the polyhedrin locus, and Rev was constructed from P35A by inserting p35 back into its native locus. Viruses were propagated in TN-368 cells and virus titres were determined in TN-368 cells by end-point dilution assays using eGFP as a marker for infected cells (O’Reilly et al., 1992). Freshly titrated stocks were used in all experiments. In experiments where caspase inhibitor was used, 100 μM N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk; MP Biomedicals) was added to the cells at the time of infection and was present throughout infection. TN-368 and SF-21 cells were cultured in TC-100 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals). To induce apoptosis in SF-21 cells, the cells were exposed to UV light by placing the culture dish on a transilluminator for 5 min.

**Caspase activity assay.** Caspase activity was determined as described previously (Muro et al., 2004) by pre-incubating the lysate with 40 μM N-acetyl-Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (Ac-DEVD-afc; MP Biomedicals) for 15 min at 37 °C. Measurements (excitation 405 nm, emission 535 nm) were obtained at 15 min intervals to determine the enzyme kinetics. All enzyme activity assays were carried out in triplicate and the results are presented as means ± SEM.

**Cell-cycle analysis.** Cell-cycle analysis was determined by flow cytometry using a FACSCalibur cytometer (Becton Dickinson) following a modified protocol (Braunagel et al., 1998; Karpf & Brown, 1998). Infections were carried out with 5 × 10⁶ cells at an m.o.i. of 5 p.f.u. per cell. At 24 and 48 h post-infection (p.i.), cells were harvested and washed twice with PBS by centrifugation at 1000 r.p.m. in a microcentrifuge for 5 min. Cells were resuspended and fixed in 70% ethanol and incubated at −20 °C overnight. The next day, the cells were washed in PBS and then resuspended in DNA stain buffer [PBS containing 0.05 mg RNase A ml⁻¹, 0.1% Triton X-100, 50 μg propidium iodide (Invitrogen) ml⁻¹, 0.1 mM EDTA, pH 7.5]. Cells were incubated in this buffer for at least 30 min before analysis. Ten thousand events were collected for each data point and data were obtained on the slow cycle of the cytometer and did not exceed 150 events s⁻¹. Doublets were excluded from analysis by analysing FL-2A (area) versus FL-2W (width), and the cell cycle was determined from this gated population of cells. AcMNPV-infected cells are arrested at the G2/M phase and also increase in area, which can be observed by an increase in FL-2A. The percentage of cells in the G2/M phase was plotted as mean ± SEM.

**Endosome exit assay.** Viral binding and entry kinetics were determined by inhibiting endosome acidification at designated time points following a previously published protocol (Hefferon et al., 1999). Briefly, 5 × 10⁵ cells were plated overnight, and the following day the cells and virus were incubated separately at 4 °C for 45 min. Infections were then carried out at an m.o.i. of 5 by incubating the chilled virus and cells together for 1 h at 4 °C. After this binding step, unbound virus was removed and warm (37 °C) medium was added to the cells, which were placed immediately in a 27 °C incubator. At 0, 30, 60, 120 and 540 min post-incubation, the medium was removed and medium containing 25 mM ammonium chloride was added to the cells. At 24 h p.i. the proportion of eGFP-positive cells was determined by flow cytometry on a FACSCalibur cytometer. Each data point represents the mean ± SEM of 10 000 events for data collected from three independent infections.

**RESULTS**

**P35A BVs are less stable and initiate infection more slowly than control BVs**

Normally, AcMNPV BV stocks are highly stable, and can be stored for years without losing significant titre, as long as
as they are protected from light (Jarvis & Garcia, 1994). However, in the past we have often observed that titres of p35 mutant BV stocks appear to decrease much faster than expected. To test this directly, we used a p35 mutant virus, P35Δ, and a control revertant virus, Rev. BV stocks of these viruses that had been produced in TN-368 cells were incubated at 30 °C in the dark for 2 months, and their titres were measured both immediately before and after the incubation period. We found that the titre of the P35Δ virus had decreased 192-fold after the 2-month incubation period, whilst the titre of Rev decreased only fivefold during this time period, suggesting that expression of P35 is somehow important for the stability of AcMNPV BVs.

In addition, while working with the P35Δ and Rev viruses, we also consistently noted what appeared to be a lower level of eGFP expression in TN-368 cells infected with P35Δ compared with Rev, even when using freshly propagated and titrated virus stocks (both viruses express eGFP from a hsp70 promoter; Clarke & Clem, 2003). This prompted us to compare more closely the kinetics of infection of P35Δ versus Rev. We first quantified eGFP expression in TN-368 cells infected with the two viruses at 24 h p.i. using flow cytometry analysis (Fig. 1a). We found that the mean fluorescence intensity of TN-368 cells infected with P35Δ was indeed significantly lower than cells infected with Rev. As the hsp70 promoter behaves as an immediate-early promoter, and as these two viruses differ only by the presence of an intact p35 gene, this suggested that P35 is important for promoting some aspect of infection at an early stage in the replication cycle.

As an additional measure of the kinetics of infection, we used an assay that takes advantage of the fact that AcMNPV infection causes cell-cycle arrest in the G2/M stage (Braunagel et al., 1998). TN-368 cells were infected with Rev or P35Δ at an m.o.i. of 5, and the cells were subjected to cell-cycle analysis by flow cytometry at 24 and 48 h p.i. As shown in Fig. 1(b), a larger proportion of cells infected with Rev were in G2/M than cells infected with P35Δ at 24 h p.i. By 48 h p.i., however, the proportion of cells in G2/M was similar with both viruses. These results indicated that the entire process of infection was delayed with the P35Δ virus, not simply expression of eGFP. The fact that there was no difference in cell-cycle arrest by 48 h p.i. indicated that the delay in P35Δ infection was only transient, and occurred at a relatively early stage of infection, prior to G2/M arrest.

To address this, we compared binding and entry of the two viruses. AcMNPV BV particles enter cells through receptor-mediated endocytosis, and the kinetics of entry can be studied using ammonium chloride, which blocks endosome acidification and virus escape from the endosome (Hefferon et al., 1999). TN-368 cells were incubated with P35Δ or Rev at an m.o.i. of 5 at 4 °C for 1 h to allow binding and then warmed to 27 °C. Ammonium chloride was then added at 0, 0.5, 1, 2, or 9 h p.i. and the infected cells were incubated at 27 °C until 24 h p.i., at which time they were assayed for eGFP expression by flow cytometry. If virus had not yet escaped from the endosome at the time of ammonium chloride addition, then eGFP expression would not be observed. It should be noted that this assay cannot discriminate between defects in virus binding versus entry. An obvious delay was observed in the binding/entry of P35Δ compared with Rev (Fig. 2). Even when ammonium chloride was added at 9 h p.i., only approximately 20 % of the P35Δ-infected cells exhibited eGFP expression at 24 h p.i., whilst the Rev virus had successfully entered approximately 45 % of the cells by 9 h p.i.

Fig. 1. The timing of P35Δ infection is slower than Rev control virus. (a) eGFP expression, measured as mean fluorescence intensity (MFI), in TN-368 cells infected with P35Δ or Rev at an m.o.i. of 5. MFI was determined at 24 h p.i. (b) Cell-cycle arrest of TN-368 cells infected with P35Δ or Rev. Cells were infected at an m.o.i. of 5 with P35Δ or Rev and harvested at 24 and 48 h p.i. In (a) and (b), the data shown are the combined results of three independent experiments and are illustrated as means ± SEM. The data were analysed by Student’s t-test and P values are indicated.
P35Δ-infected TN-368 cells contain active caspases

As the only well-described function of P35 is its ability to inhibit caspases, we hypothesized that caspases were perhaps being activated in P35Δ-infected TN-368 cells, and that these caspases were somehow damaging the P35Δ virions, either during or after their assembly in cells. Although TN-368 cells infected with p35 mutant viruses do not become apoptotic (Clem et al., 1991; Griffiths et al., 1999; Hershberger et al., 1992), to our knowledge it has not previously been determined whether caspases are activated in TN-368 cells upon infection with viruses lacking p35. Kelly et al. (2006) observed caspase activation in TN-368 cells infected with a virus known as AcdefrT, but this virus contained mutations in both p35 and the fp-25 gene, and caused an unusual phenotype in TN-368 cells that somewhat resembled apoptosis.

To measure caspase activity, TN-368 cells were infected with either P35Δ or Rev, and cells were harvested at 24 and 48 h p.i. and assayed for the ability to cleave the fluorogenic caspase substrate Ac-DEVD-afc, which is recognized mainly by effector caspases (Fig. 3a). Cells infected with Rev showed no detectable caspase activity at either time point, and actually had lower caspase activity than mock-infected cells, presumably due to expression of P35 (Fig. 3a). Cells infected with P35Δ, on the other hand, exhibited higher amounts of caspase activity than mock-infected cells, and the amount of activity increased from 24 to 48 h p.i. (Fig. 3a). Thus, even though P35Δ-infected TN-368 cells do not exhibit morphological signs of apoptosis, some amount of caspase activation still occurs in these cells.

To gain a better appreciation of the relative level of caspase activation in P35Δ-infected TN-368 cells, we wanted to compare this level of caspase activity with that in cells that were actually going through apoptosis. As TN-368 cells are highly resistant to apoptosis, we used SF-21 cells, which are sensitive to a variety of apoptotic stimuli. SF-21 cells were exposed to UV light and harvested at 3 and 5 h

**Fig. 2.** Endosome exit assay of P35Δ and Rev in TN-368 cells. TN-368 cells were infected at an m.o.i. of 5 and ammonium chloride was added at 0, 0.5, 1, 2 and 9 h p.i. to block endosome acidification. At 24 h p.i., the proportion of infected cells (measured by eGFP expression) was determined. The 24 h time point represents cells that were not treated with ammonium chloride. The data shown are the combined results of three independent experiments and are illustrated as means ± SEM. The two datasets were found to be statistically different as analysed by two-way analysis of variance (P<0.0001).

**Fig. 3.** Caspase activity in infected TN-368 cells. (a) TN-368 cells were infected with P35Δ or Rev at an m.o.i. of 5 and harvested at 24 and 48 h p.i. Virus-infected and mock-infected lysates were incubated with the fluorogenic caspase substrate Ac-DEVD-afc to measure effector caspase activity. Following a 15 min pre-incubation with substrate, fluorescence was measured every 15 min for 75 min. (b) Comparison of P35Δ-induced caspase activity in TN-368 cells with caspase activity in SF-21 cells treated with UV to induce apoptosis and harvested at 3 or 5 h post-UV treatment. One-third of the amount of lysate was used in (b) compared with (a). For both panels, the data shown were obtained from three independent experiments and are plotted as means ± SEM. Data were analysed by two-way analysis of variance (P<0.0001).
post-exposure. By 3 h post-UV exposure, caspases were activated in SF-21 cells and the cells began to show morphological characteristics of apoptosis such as plasma membrane blebbing (data not shown and Manji & Friesen, 2001). We then compared the level of caspase activity in UV-treated SF-21 cell lysates with lysates harvested from the same number of P35Δ- or Rev-infected TN-368 cells at 48 h p.i. (Fig. 3b). Because the amount of caspase activity in the UV-treated SF-21 cells was so high, we used only one-third of the amount of lysate used in Fig. 3(a) for all of the samples. SF-21 cells treated with UV had much higher caspase activity than P35Δ-infected TN-368 cells; the activity of the UV-treated SF-21 cells reached a plateau after only a short time, indicating that the substrate in the reaction had been exhausted. It was difficult to make a direct comparison between the different cell lines, but the amount of caspase activity in P35Δ-infected TN-368 cells appeared to be considerably lower than that in apoptotic SF-21 cells. Interestingly, untreated SF-21 cells had lower levels of caspase activity than mock-infected TN-368 cells (Fig. 3b), suggesting that TN-368 cells may contain a low level of constitutive caspase activity.

Inhibition of caspases in cells producing P35Δ rescues the entry defect in the next round of replication

To test whether the observed caspase activity in P35Δ-infected cells was involved in the entry defect of P35Δ, we used the chemical pan-caspase inhibitor zVAD-fmk. We considered two possibilities for when and where the activation of caspases could be having a detrimental effect on P35Δ infectivity: either in the cells used to produce the P35Δ virus stocks (the producer cells), in which case the virions would already be defective at the start of the next round of infection, or during the actual process of infection of the cells used to measure infectivity (the target cells). To test the first possibility, viruses were propagated in the presence of zVAD-fmk to inhibit caspase activation in the producer cells, and these viruses were then analysed for their infection kinetics. When eGFP expression was analysed using viruses produced in the presence of zVAD-fmk, we found that the reduction in eGFP expression seen for P35Δ (Fig. 1a) was rescued (Fig. 4a). In addition, the P35Δ virus propagated in the presence of zVAD-fmk also had a normal binding/entry phenotype when used to infect TN-368 cells (Fig. 4b).

To test the second possibility, P35Δ or Rev stocks that had been produced in cells without caspase inhibitor were used to infect TN-368 cells in the presence of zVAD-fmk and cell-cycle analysis was carried out to measure G2/M arrest (Fig. 5). The results were very similar to infections carried out in the absence of zVAD-fmk (Fig. 1b); there was a delay in cell-cycle arrest at 24 h p.i. in P35Δ-infected cells compared with Rev-infected cells, but this was no longer evident by 48 h p.i. Together, these results indicated that caspases are responsible for the decreased infectivity of P35Δ BVs, and that the detrimental effect of caspases on the infectivity of P35Δ BVs occurs prior to the next round of replication.

DISCUSSION

Apoptosis has been shown to serve as an antiviral defence for many viruses because of its negative effects on the process of virus replication. This is the first report, as far as
Caspase activity leads to defective baculovirus virions

we are aware, that illustrates that caspase activity can also have detrimental effects on progeny virus particles and affect their subsequent infectivity in other cells. These effects thus represent an additional, previously unrecognized, facet of the antiviral apoptotic response. Our results indicate that activated caspases in the cells that produce the progeny virus are responsible for the infectivity defect of P35Δ virions, as entry was restored to normal by treating the cells used to produce the virus stocks with the caspase inhibitor zVAD-fmk. The relative instability of P35Δ virus stocks further indicates that caspases can directly or indirectly have negative effects on virions and their ability to initiate infection. Activated caspases could affect virions during or after their assembly, either by cleaving a cellular substrate that the virus needs for efficient packaging or by directly cleaving viral proteins. Another possibility is that viral DNA may be damaged by the nucleases that are activated by caspases and digest cellular DNA during apoptosis. We consider this unlikely, however, as p35 mutant-infected TN-368 cells do not undergo apoptosis or exhibit DNA degradation (Clem et al., 1991) and viral DNA is not degraded even when SF-21 cells are infected with p35 mutant AcMNPV (R. J. Clem and L. K. Miller, unpublished results).

This is the first report demonstrating that caspase activation, albeit at a low level, occurs in TN-368 cells infected with the p35 mutant AcMNPV. This result was somewhat surprising, as TN-368 cells are resistant to most apoptotic stimuli. It has been shown that caspases are activated in TN-368 cells infected with a virus containing mutations in both p35 and fp-25, but such cells die by a process that appears to have some of the characteristics of apoptosis (Kelly et al., 2006). The mechanism responsible for the resistance of TN-368 cells to apoptosis is unknown, but, interestingly, our results indicate that uninfected TN-368 cells have a low level of caspase activity, which is not seen in SF-21 cells. Mamestra brassicae and Panolis flammea cell lines have also been shown to support replication of the p35 mutant AcMNPV (Griffiths et al., 1999). Given our results, it would be interesting to determine whether these cell lines also activate caspases in response to infection.

It has been concluded previously that no significant differences are observed between the p35 mutant and wild-type AcMNPV infection in TN-368 cells or T. ni larvae (other than the lack of larval liquefaction). However, when one closely examines the previously published data, our results are actually consistent with results from some of these previous studies both at the organism level and at the cellular level. Data published by Clarke & Clem (2003) suggested a possible slower spread of the P35Δ virus than Rev in T. ni larvae, as there was a somewhat lower proportion of haemocytes infected with P35Δ compared with the Rev virus following intrahaemocoelic infection. This difference was seen at 24 and 48 h p.i., although the difference was not statistically significant at 24 h p.i., and by 72 h p.i. the proportions of infected haemocytes were similar between the two viruses. At the cellular level, Clem & Miller (1993) reported differences in virus replication and viral gene expression in SF-21 cells infected with two different p35 mutants and wild-type virus, but no differences were reported in TN-368 cells. However, even though it was not addressed in the report, it appears that there were also slightly lower levels of transcripts for the late vp39 and the very late polh genes in TN-368 cells infected with p35 mutant virus compared with wild-type, although transcript levels of an early gene appeared similar. However, Hershberger et al. (1992) reported similar levels of late and very late gene expression for p35 mutant and control viruses in TN-368 cells. The basis for the differences between these reports is unknown, but Hershberger et al. (1992) also noticed a difference in infectivity between viruses containing or lacking p35. These authors reported that, following infection with p35 mutant virus at an m.o.i. of 5, a large proportion of SF-21 cells did not exhibit cytopathic effects, unlike control viruses, which caused cytopathic effects in nearly all cells at the same m.o.i. As the same effect was seen when virus was propagated in TN-368 or SF-21 cells, the authors concluded that it was not due to apoptosis, and postulated that P35 had a direct role in infectivity. The same group later showed that P35 protein is associated with purified BV particles, suggesting that P35 might be a component of the virion, necessary either for virus stability (as a
structural protein) or for promoting infection in some way, such as inhibiting caspases during the early stages of infection (Hershberger et al., 1994). Our results, however, appear to rule out these possibilities, at least for TN-368 cells. We observed that zVAD-fmk treatment of the cells used to propagate the virus rescued the infectivity defect of progeny P35Δ virus, whilst the infectivity of P35Δ was not rescued by the addition of the inhibitor during subsequent infection, indicating that inhibition of caspases is not necessary during the early stages of infection in TN-368 cells. The situation could be different in SF-21 cells, however, which have higher levels of caspase activity following P35Δ infection.

We suggest that the detrimental effect of caspases on BV infectivity may also occur during replication of the p35 mutant AcMNPV in T. ni larvae, and that this could potentially explain the lack of liquefaction in these larvae. As progeny virus is produced in the tissues of T. ni larvae, defects in its ability to infect neighbouring cells would result in less efficient spread of infection throughout the organism and possibly lower levels of expression of viral genes such as chitinase and cathepsin, which are known to be required for liquefaction (Hawtin et al., 1997; Ohkawa et al., 1994; Slack et al., 1995).

The ability of P35 to protect progeny virions from caspase-induced damage is somewhat analogous to the role of the human immunodeficiency virus type 1 (HIV-1) protein Vif in inhibiting the cellular enzyme APOBEC3G. Vif is packaged in the HIV-1 capsid and, during the subsequent round of replication, binds to APOBEC3G, a cytosine deaminase that introduces mutations into the viral genome and targets it for destruction by ubiquitination (Harris & Liddament, 2004). Like Vif, P35 inhibits cellular enzymes that can potentially cause damage to virions. Whether P35 needs to be packaged in the virion to carry out this aspect of its function, and whether the damage is due to direct cleavage of viral structural proteins or to an indirect effect on virus assembly, remain open questions.

In this report, we have shown that caspases are activated upon AcMNPV infection in TN-368 cells from the host T. ni, even though these cells do not die by apoptosis. Based on prior studies, it had been thought that P35 is not necessary for replication in T. ni cells and larvae, but our results indicate that even a low level of caspase activity can have detrimental effects on the infectivity of baculovirus virions. This potentially represents a previously unappreciated consequence of the anti-apoptotic response against viruses. It will be interesting to determine whether other types of virus are also negatively impacted by caspase activation during apoptosis of their host cells.

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