Prevention of baculovirus-induced apoptosis of BTI-Tn-5B1-4 (Hi5) cells by the p35 gene of Trichoplusia ni multicapsid nucleopolyhedrovirus

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A typical apoptosis of BTI-Tn-5B1-4 (Hi5) cells induced by Heliothis armigera single capsid nucleopolyhedrovirus (HaSNPV) infection was completely suppressed by coinfection with Trichoplusia ni multicapsid nucleopolyhedrovirus polyhedron-negative recombinant (TnMNPV-SV17G) (OCC) at a low multiplicity of infection (6.5 × 10⁻²). To determine whether TnMNPV p35 alone was sufficient to inhibit the apoptosis, two recombinant plasmids containing the early promoter of p35, or the very late promoter of TnMNPV polh were constructed to study p35 function by transient expression assay. It was shown that expression of p35 alone could partially prevent HaSNPV-induced apoptosis but did not facilitate HaSNPV replication in Hi5 cells. The data suggests that both P35 of TnMNPV and other unknown gene products are required for the suppression of apoptosis and facilitation of HaSNPV replication in Hi5 cells.

In baculoviruses, the products of two viral genes, p35 and iap, are involved in the inhibition of apoptosis (Teodoro & Branton, 1997; Miller, 1997). The p35 gene product is unique for this virus group, inhibiting not only baculovirus-induced apoptosis in insect cells (Clem et al., 1991) but also developmentally programmed cell death in the nematode Caenorhabditis elegans (Sugimoto et al., 1994) and Drosophila flies (Hay et al., 1994). In addition, P35 can prevent neuronal cell death induced by serum or nerve growth factor deprivation in mammalian cells (Rabizadeh et al., 1993; Martinou et al., 1995) and apoptotic cell death induced by Fas and tumour necrosis factor (Beidler et al., 1995). Thus, it seems the function of P35 is highly conserved in evolution. The p35 promoter of Autographa california multicapsid nucleopolyhedrovirus (AcMNPV) has both early and late regulating elements that direct early and late mRNA synthesis (Nissen & Friesen, 1989; Dickson & Friesen, 1991). P35 protein is first detected at 8–12 h post-infection (p.i.) and accumulates through later stages of infection (24–36 h p.i.) (Hershberger et al., 1992, 1994). P35 protein is found predominantly in the cytosol (Hershberger et al., 1994), unlike the mammalian anti-apoptotic protein BCL-2, which is localized mainly in the membranes of the mitochondria, endoplasmic reticulum or nucleus (Hockenbery et al., 1990). In addition to its role as an inhibitor of apoptosis, P35 can stimulate baculovirus DNA replication (Kool et al., 1994), increase transcription from the 39 kDa promoter of AcMNPV (Gong & Guarino, 1994; Gong et al., 1998) and promote transformation of mouse embryonic fibroblasts (Resnoff et al., 1998).

A second class of baculovirus genes, iaps (inhibitors of apoptosis), can be found also in both insect viruses and mammals (Crook et al., 1993; Birnbaum et al., 1994). These genes encode a C3HC4 (or RING) finger motif found in a number of transcriptional regulatory proteins, as well as two additional novel Cys/His motifs called baculovirus iap repeats (BIRs) (Crook et al., 1993; Birnbaum et al., 1994). Specific amino acids within the C3HC4 finger and the N-terminal BIR are critical for IAP anti-apoptosis function (Clem & Miller, 1994). It is believed that the mechanism by which the p35 product inhibits apoptosis differs from that of the iaps products. While P35 prevents apoptosis by specifically inhibiting CED-3/ICE death protease (caspase I), the iaps proteins function at an earlier stage in the pathway, probably involving other caspases in addition to caspase 1 (Seshagiri et al., 1997; Vucic et al., 1997; Manji et al., 1997; Bertin et al., 1996; Ahmad et al., 1997).

In China, Heliothis armigera single nucleocapsid nucleopolyhedrovirus (HaSNPV) was first described in 1978 (Cai, 1978) and was later developed as the first commercial insecticide against the cotton bollworm in more than 20 provinces (Li & Pang, 1990; Zhang et al., 1994). Restriction fragment length polymorphism in several HaSNPV isolates...
HaSNPV at an m.o.i. of 5 and TnMNPV-SVI apoptosis was partially inhibited when Hi5 cells were coinfected with HaSNPV at an m.o.i. of 5 and TnMNPV-SVI.

To our surprise, when challenged with HaSNPV, the HaSNPV Hubei strain (Cai, 1978) was provided by Zhihong Hu (Institute of Virology, Academia Sinica, Wuhan, People’s Republic of China). TnMNPV polyhedron-negative recombinant virus TnMNPV-SVI-G (OCC°) was constructed in our laboratory (Wang et al., 1991). TnMNPV (Kelly & Lescott, 1981) was donated by D. C. Kelly (NERC Institute of Virology and Environmental Microbiology, Oxford, UK) and has a very similar genome and protein structure to AcMNPV (Miller & Dawes, 1978; Wang & Kelly, 1983). Trichoplusia ni cell line BTI-Tn-5B1-4 (Hi5) (Granados et al., 1994) was donated by R. R. Granados (Booye Thompson Institute, USA) and Heliothis zea cell line Hz-IB3 (McIntosh & Ignoffo, 1983) by Quhou Chen (Central China Normal University, People’s Republic of China). All cells were propagated at 27°C in TC-100 medium supplemented with 10% foetal bovine serum (Gibco). TnMNPV-SVI-G was replicated in Hi5 while HaSNPV was replicated in Hz-IB3 cells. The virus titre was determined by plaque assay as previously described (O’Reilly et al., 1992). Isolation of apoptotic DNA fragments was as described by Herrmann et al. (1994).

Hi5 cells were coinfected with HaSNPV (at an m.o.i. of 5) and TnMNPV-SVI-G (at an m.o.i. of 5). As a control, Hi5 cells were infected with HaSNPV alone at an m.o.i. of 5. Light microscopic observations and the presence or absence of DNA ladders indicated that the cells coinfected with HaSNPV and TnMNPV-SVI-G did not undergo apoptosis even by 72 h p.i. (Fig. 1a). In these cells, polyhedra (OCC°) were detected in about 30% of the coinfected cells 72 h p.i., while the other 70% of cells showed typical virus-infected cytopathic effect with a polyhedron-negative phenotype (OCC−). Since the TnMNPV recombinant virus TnMNPV-SVI-G (OCC−) was used in the coinfection, the presence of polyhedra was due to spread of the HaSNPV. The supernatant from the coinfected cells was infective to both Hz-IB3 and Hi5 cells (data not shown), indicating that coinfection of HaSNPV and TnMNPV-SVI-G not only inhibited completely HaSNPV-triggered apoptotic cell death but also facilitated HaSNPV replication in Hi5 cells. In the HaSNPV-infected Hi5 cells, apoptosis appeared at 24 h p.i. with a peak of apoptotic mortality (approx. 60% of cells) at 48 h p.i. (Fig. 1a). Although a few viral polyhedra were observed in 5% of cells, the supernatant of the culture was not infective to Hz-IB3 cells.

To determine the minimum quantity of TnMNPV-SVI-G required for inhibiting HaSNPV-induced apoptosis of Hi5 cells, Hi5 cells were coinfected with HaSNPV (at an m.o.i. of 5) and TnMNPV-SVI-G at increasing m.o.i. (5–6·5 × 10⁻⁵). Fig. 1(b) shows that an m.o.i. for TnMNPV-SVI-G as low as 6·5 × 10⁻² was sufficient for complete inhibition of apoptosis and the facilitation of HaSNPV replication in Hi5 cells.

To determine whether the TnMNPV P35 alone was sufficient to inhibit the apoptosis, we constructed two p35 plasmids to study P35 function by transient expression assay. The two p35 plasmids were constructed as follows. According to the reported AcMNPV p35 sequence (Friesen & Miller, 1987), we amplified the p35 early promoter region and its open reading frame (p35_EPO) from the TnMNPV genome using.

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**Fig. 1.** (a) Prevention of HaSNPV-induced apoptosis of Hi5 cells by TnMNPV-SVI-G. M, Molecular mass marker (λDNA/EcoRI + HindIII); A, uninfected Hi5 cells; B, Hi5 cells coinfectd with TnMNPV-SVI-G and HaSNPV at 72 h.p.i., showing complete inhibition of HaSNPV-induced apoptosis of Hi5 cells by TnMNPV-SVI-G; C, Hi5 cells infected with TnNPV-SVI-G at an m.o.i. of 5, 48 h p.i., no apoptosis observed; D, Hi5 cells infected with HaSNPV at an m.o.i. of 5, 48 h p.i., showing the characteristic DNA ladder. (b) Dosage–effect analysis of TnMNPV-SVI-G for prevention of HaSNPV-induced apoptosis in Hi5 cells. HaSNPV-induced apoptosis was completely inhibited when Hi5 cells were coinfectd with HaSNPV at an m.o.i. of 5 and TnMNPV-SVI-G at an m.o.i. of 6·5 × 10⁻¹ or 6·5 × 10⁻²; HaSNPV-induced apoptosis was not inhibited when Hi5 cells were coinfectd with HaSNPV at an m.o.i. of 5 and TnMNPV-SVI-G at an m.o.i. of 6·5 × 10⁻³; HaSNPV-induced apoptosis was partially inhibited when Hi5 cells were coinfectd with HaSNPV at an m.o.i. of 5 and TnMNPV-SVI-G at an m.o.i. of 6·5 × 10⁻¹.
Hi5 cells were transfected using the p35 plasmid DNA as described by Kool et al. (1993). A 35 mm tissue culture plate was seeded with 2 \times 10^5 Hi5 cells and incubated at 27 °C overnight to allow the cells to attach. Five µg of pUC-p35\textsuperscript{EPO} or pX3-p35\textsuperscript{EPO} plasmid DNA and 20 µl of Lipofectamine reagent (Gibco BRL) were transfected into the Hi5 cells. After incubation at 27 °C for 6 h, the DNA-containing medium was replaced by 1.5 ml of fresh medium, and the cells were incubated at 27 °C for 24 h. The cells were then infected with HaSNPV at an m.o.i. of 5. As a control, 2 \times 10^5 cells were transfected with equimolar amounts of the pUC-18 or pX3-p35\textsuperscript{EPO} plasmids, and then infected with HaSNPV at an m.o.i. of 5, showing partial inhibition of HaSNPV-induced apoptosis by pUC-p35\textsuperscript{EPO} in Hi5 cells.

In our constructs, the two plasmids above did not show significant differences in the inhibition of HaSNPV-induced apoptosis in Hi5 cells, meaning that late expression of p35 in this case did not further suppress apoptosis.

When the cells were transfected with increasing quantities of plasmid pUC-p35\textsuperscript{EPO} (0.5–10 µg DNA per 2 \times 10^5 Hi5 cells) and then infected with HaSNPV at an m.o.i. of 5 at 24 h after transfection, the results showed that transfection of 2 \times 10^5 Hi5 cells with 2–5 µg of plasmid pUC-p35\textsuperscript{EPO} resulted in significant suppression of apoptosis (Fig. 2b).

Taken together, our results indicated that the p35 product of TnMNPV is able to prevent HaSNPV-induced apoptosis of Hi5 cells; both the p35 gene product and other unknown gene products are required to completely suppress the apoptosis and, at the same time, facilitate HaSNPV replication in Hi5 cells.

It is generally believed that apoptosis provides cells with an effective and final antiviral defence mechanism to prevent virus infections, and suppression of apoptosis by the expression of anti-apoptotic genes is expected to promote the replication of viruses and increase the yield of virus progeny (Teodoro \& Branton, 1997; Miller, 1997). It had been shown that early expression of P35 at a low level is sufficient to block apoptosis, while late expression of P35 is required to maintain wild-type virus gene expression and facilitate virus replication (Hershberger et al., 1992, 1994). Thus, P35 acts as a host range determinant by suppressing the ability of the infected cells to undergo apoptosis. However, it had been reported that P35 may not be the only host range determinant in the AcMNPV–S. littoralis (SL2) cell system, since it inhibits AcMNPV-induced apoptosis of SL2 cells but does not boost the yield of budded AcMNPV (Gershburg et al., 1997). In the present study, our data also showed that expression of P35 could partially inhibit HaSNPV-induced apoptosis in Hi5 cells but does not facilitate HaSNPV replication.
Coinfection of Hi5 cells with HaSNPV and TnMNPV-SVI-G resulted in replication of HaSNPV. There are two possibilities: (i) a helper function of TnMNPV-SVI-G gene products facilitates HaSNPV replication in non-permissive Hi5 cells, or (ii) recombinant viruses between HaSNPV and TnMNPV-SVI-G were produced. We tried hard but eventually failed to construct a p35–HaSNPV recombinant virus using HaSNPV and pX3-p35_EPO, which contains the flanking sequences of the TnMNPV polh (data not shown), suggesting that the polh flanking sequences of HaSNPV and TnMNPV are quite different. Also, we were unable to obtain a recombinant virus between TnMNPV-SVI-G and HaSNPV by the plaque purification method (data not shown). Thus, it is very possible that TnMNPV gene product(s) facilitate HaSNPV replication in Hi5 cells.

In addition, we were unable to identify a TnMNPV p35 homologue in the genome of HaSNPV by Southern blotting (data not shown), which could be due to the absence of p35 in the HaSNPV genome or could indicate low identity between TnMNPV p35 and a functional homologue of p35 in HaSNPV.

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References


Granados, R. R., Li, G. X., Derksen, A. C. G. & McKenna, K. A. (1994). A new insect cell line from Trichoplusia ni (BTI-Tn-5B1-4) susceptible to Trichoplusia ni single enveloped nuclear polyhedrosis virus. Journal of Invertebrate Pathology 64, 260–266.


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