The prototype baculovirus, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) expresses p35, a potent anti cell-death gene that promotes the propagation of the virus by blocking host cell apoptosis. Infection of insect SF-21 cells with AcMNPV lacking p35 induces apoptosis. We have used this pro-apoptotic property of the p35 null virus to screen for genes encoding inhibitors of apoptosis that rescue cells infected with the p35 defective virus. We report here the identification of Tn-IAP1, a novel member of the IAP family of cell death inhibitors. Tn-IAP1 blocks cell death induced by p35 null AcMNPV, actinomycin D, and *Drosophila* cell-death inducers HID and GRIM. Given the conserved nature of the cell death pathway, this genetic screen can be used for rapid identification of novel inhibitors of apoptosis from diverse sources.

Apoptosis or programmed cell death plays a vital role in the normal development and homeostasis of metazoans (1–3). Defective programmed cell death has been linked to a variety of human diseases including cancer, neurodegeneration, and autoimmune disorders (3). Additionally, apoptosis serves as an important host defense mechanism against invading pathogens, wherein infected cells undergo programmed cell death thereby preventing propagation and spread of the pathogen (4, 5). To antagonize host defense mechanisms, pathogens have in turn evolved ways to attenuate the host apoptotic pathway (4, 5).

Baculoviruses, a family of arthropod-specific double-stranded DNA viruses, were one of the first viruses shown to elicit and modify the host apoptotic response (6). AcMNPV is the prototype baculovirus used to study host-pathogen apoptotic interactions. AcMNPV infects SF-21 cells, a cell line derived from the fall army worm *Spodoptera frugiperda*, which results in the production of budded virus (BV) early during infection and occluded virus (OV) starting 24 h postinfection (6, 7). Propagation of AcMNPV in SF-21 cells proceeds normally because of the expression of P35, a virally encoded pan-caspase inhibitor (8–10). Infection of SF-21 cells with a p35 null mutant of AcMNPV results in widespread apoptosis leading to reduced BV and no OV production (7, 11).

Induction of apoptosis by p35 mutants of AcMNPV in SF-21 cells can be blocked by viral IAPs (inhibitors of apoptosis) (7, 12, 13). The IAPs are a family of cell death inhibitors found in both vertebrates and invertebrates and are known to block cell death induced by various stimuli (14–16). The first members of the IAP family, Op-iap and Cp-iap, were isolated from the genome of two baculoviruses CpGV and OpNPV by their ability to substitute for p35 in AcMNPV (12, 13).

The conserved nature of the apoptotic machinery across the animal kingdom allows for the use of AcMNPV as a powerful genetic tool in identification of genes that block apoptosis. We describe the use of a p35 null mutant of AcMNPV to screen expression cDNA libraries for anti-apoptotic genes. Using this system we identified TnIAP1, a novel IAP, from TN368-derived cDNA libraries. In contrast to SF-21 cells, TN368 cells, derived from the cabbage looper *Trichoplusia ni*, support the normal growth of p35 null mutant of AcMNPV, leading to the production of both the BV and OV (6, 11). The ability of TN368 cells to support the propagation of p35 null AcMNPV implied the presence of an endogenous inhibitor of apoptosis that effectively substitutes for p35 function. Identification of such an endogenous inhibitor using TN368 expression cDNA libraries and p35 null AcMNPV permitted a stringent test of the general applicability of the system.

### EXPERIMENTAL PROCEDURES

**Cell Lines and Virus—**Fall army worm *Spodoptera frugiperda* (Lepidoptera: noctuidae)-derived IPLB-SF-21 (SF-21) cells were maintained in TC-100 medium (Sigma) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) as described previously (17, 18). TN368 cells, derived from the cabbage looper *T. ni*, were also maintained in TC-100 medium.

**vP53del,** a p35 null mutant of AcMNPV was cultured and titered in TN368 cells (7), and the viral DNA was prepared as described (17).

**cDNA Library—**Oligo(dT)-primed cDNA library was made in a modified pHSP70PLVI†+CAT (7) plasmid using mRNA derived from TN368 cells. pHSP70PLVI+CAT, a previously described plasmid (7), was modified to create 5′ XhoI and 3′ NotI sites flanking the gene for CAT (chloramphenicol acetyltransferase) such that it could be replaced with cDNAs of interest. Expression cDNAs in this vector are under the control of the *Drosophila* 70-kDa heat-shock protein (hsp70) promoter. The expression cassette is flanked by AcMNPV sequences that mediate homologous recombination allowing for the integration of the expression cassette into the viral genome, thereby creating a recombinant virus expressing the cDNA of interest.

**Plasmid Constructs—**All plasmids used in this study were constructed in their pHSP70PLVI+CAT backbone using CAT with an appropriate insert within the BglII (5′) and NotI (3′) sites. The various TN-IAP1 constructs used in this study contained an in-frame HA.11 epitope tag (YPYDVPDYA) at the N terminus. PhsTn-IAP1-FLVI+ contains (residues 1–379) the complete open reading frame encoding Tn-IAP1, phsTn-IAP1-FL-17VI+ encodes for Tn-IAP1 lacking the first 17 residues, phsTn-IAP1-BIRsVI+ codes for the baculovirus IAP re-
Viral Genetic Screen Identifies a Novel IAP

Use of p35 Null Baculovirus to Identify Anti-cell Death Genes—We have developed a genetic screen to identify novel anti-cell death genes present in expression cDNA libraries. Upon infecting Sf-21 insect cells, vP35del virus induces widespread apoptosis leading to greatly reduced BV production and no OV production (7). In contrast, the ability of the cabbage looper-derived cell line TN368 to support normal propagation of vP35del virus suggested that this cell line potentially expressed an inhibitor of apoptosis.

When supplied in trans, any gene that can block vP35del-induced cell death will lead to the production of OV and restore the levels of BV produced. To screen for potential anti-cell death genes present in TN368 cells, we constructed an expression cDNA library in a transfer plasmid containing a SF21-permissible hsp70 promoter (“Experimental Procedures”). This vector allowed for homologous recombination-mediated integration of the expression cassette into the vP35del genome and allowed production of a library of recombinant viruses (Fig. 1A).

Apoptosis-susceptible Sf-21 cells were co-transfected with the TN368-derived cDNA library and vP35del viral DNA and screened for production of OV. At 8 days post-transfection, production of OV was observed in cell cultures transfected with the cDNAs of OV was observed in cell cultures transfected with the cDNAs of TN368 DNA, whereas cells transfected with control plasmid encoding CAT showed no signs of productive infection (Fig. 1B, data not shown). The rescued viruses from productive infections were plaque-purified and characterized by sequence analysis. Of 25 rescued viruses, 6 contained a 2.6-kilobase cDNA encoding Tn-IAP1 that lacked the N-terminal 17 amino acids.

Tn-IAP1 Is Closely Related to Viral IAPs—The 2.6-kilobase cDNA encoding Tniap1, which contains an in-frame deletion that removes the region coding for BIR2 (residues 200–379), contains a truncated cDNA encoding TnIAP1 that lacked the N-terminal 17 amino acids.
cDNA isolated for its anti-cell death activity contained an open reading frame of 1.14 kilobases that encoded an IAP homolog very similar to the viral IAPs, Op-IAP and Cp-IAP (Fig. 2B).

Tn-IAP1 was most similar to CpIAP with 61% identity but had a longer N terminus compared with known viral IAPs (Fig. 2B). The IAP family of genes are characterized by the presence of BIR motifs at the N-terminal end of the protein and a RING finger at the C-terminal end (14). Whereas the number of BIR motifs present in the various IAPs varies between one and three, the anti-apoptotic baculovirus IAPs typically contain two BIRs. Tn-IAP1, although cellular in origin, like the baculovirus virus IAPs contains two N-terminal BIRs and a C-terminal RING finger. Multiple sequence alignments of the Tn-IAP1 and baculovirus IAPs showed Tn-IAP1 to be more similar to the anti-apoptotic viral IAPs (Op-IAP and Cp-IAP) than to other viral IAPs (Ac-IAP and Op-IAP1) whose function remains to be elucidated (Fig. 2B).

Tn-IAP1 BIRs and RING Are Required to Block Virus or Actinomycin D-induced Cell Death—To confirm the anti-apoptotic activity of the open reading frame encoding Tn-IAP1, we tested it for its ability to block vP35del-induced apoptosis. Plasmid expressing Tn-iap1 open reading frame or an N-terminal 17-amino acid deletion variant of Tn-IAP1, under hsp70 promoter control, when co-expressed with vP35del viral DNA was able to complement the p35 defect in the virus and supported the production of OV at 3–4 days postinfection (Fig. 3A).

However, plasmids expressing the two BIRs or the RING or a combination of either one of the BIRs with the RING finger when co-transfected with the vP35del viral DNA did not lead to the production of OV (Fig. 3A). These results indicate that both the BIRs and the RING finger are necessary for Tn-iap1 to block virus-induced apoptosis and promote normal replication of p35 null AcMNPV.

Actinomycin D is a potent chemical inducer of apoptosis in SF-21 cells (7). It has been shown that transient expression of Op-IAP or Cp-IAP or P35 can block ActD-induced apoptosis of SF-21 cells. We determined whether Tn-IAP1 could similarly block ActD-induced apoptosis. Full-length Tn-IAP1 or the N-terminal deletion (FL-17) protected roughly 40% of the cells from undergoing apoptosis induced by ActD (Fig. 3B). However, the expression of the BIRs or the RING or a combination of BIR1-RING or BIR2-RING did not block apoptosis induced by ActD (Fig. 3B). These results show the requirement of both BIRs and RING for the activity of Tn-IAP1 against ActD-induced cell death. The requirement of both BIRs and RING for the anti-apoptotic activity of Tn-IAP1 is consistent with similar findings reported for the baculovirus IAPs, Op-IAP and Cp-IAP (7).

BIR2 Is Necessary to Block HID- and GRIM-induced Apoptosis—We investigated whether Tn-IAP1, like Drosophila D-IAP1 and D-IAP2 and baculovirus Op-IAP and Cp-IAP, can block HID- and GRIM-induced apoptosis. To test this, SF-21 cells were cotransfected with plasmids expressing HID or

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**Fig. 2.** A, the complete nucleotide sequence of Tn-IAP1 with the putative open reading frame are shown. The BIRs and the RING finger motifs present within Tn-IAP1 are **underlined.** B, ClustalW multiple sequence alignment of Tn-IAP1 and related baculovirus IAPs. The alignments were performed using MacVector with gap distance of 6, open gap penalty of 2.1, extend gap penalty of 0.2, and the blosum similarity matrix. The GenBank® accession numbers of sequences used for the alignment are: AF195528 (Tn-IAP1), P41436 (Op-IAP), P41437 (Op-IAP), ACC59040 (Op-IAP1), and P41435 (Ac-IAP).
Because Tn-IAP1 FL-17 was found to be as effective as FL in blocking apoptosis and because it was detected at higher levels on Western blots, we used Tn-IAP1–17 and its derivatives for further studies. Tn-IAP1 FL-17 when co-expressed with HID or GRIM was effective in reducing the induction of cell death by 4-fold compared with the CAT control (Fig. 3, C and D). We also found Tn-IAP1 FL-17 to be effective at blocking RPR-induced apoptosis in Sf-21 cells (data not shown). We further tested if the BIRs or RING domains would be sufficient for inhibiting HID- or GRIM-induced apoptosis. In contrast to their ability to block virus or ActD-induced apoptosis, constructs expressing both the BIRs or the BIR2 and RING efficiently reduced HID- and GRIM-induced apoptosis by 3–4-fold (Fig. 3, C and D). However, neither the RING finger domain nor the BIR2-deleted form of Tn-IAP1, BIR1RING, was able to block HID- or GRIM-induced apoptosis (Fig. 3, C and D). The ability of BIRs or the BIR2RING to block the HID- and GRIM- and not virus- or ActD-induced apoptosis could be in part related to the potency of these different inducers of cell death. Expression of the BIR domains of Drosophila D-IAP1 also inhibited HID-induced apoptosis and served as a positive control (Fig. 3C). These results show that Tn-IAP1 inhibits HID- and GRIM-induced apoptosis in Sf-21 cells. Although the ability of BIR2 alone in blocking HID- and GRIM-induced cell death remains to be determined, the deletion of BIR2 from Tn-IAP1 abrogates its anti-cell death activity.

Baculovirus and Drosophila IAPs are known to physically interact with and inhibit RPR-, HID-, and GRIM-induced cell death (19, 21, 24). To test if Tn-IAP1 might similarly interact with these Drosophila pro-cell death proteins, we co-expressed GD-tagged GRIM with HA-epitope-tagged Tn-IAP1 constructs and determined if the two molecules physically associated. D-IAP1 served as a positive control (Fig. 4A). The Tn-IAP1 construct Tn-IAP1–17, containing both the BIRs and RING, co-precipitated with GRIM (Fig. 4A), HID, and RPR (data not shown). Additionally, various domains of Tn-IAP1 were tested for their interaction with GRIM. The BIRs of Tn-IAP1 and BIR2RING co-precipitated with GRIM, whereas neither the RING finger nor the construct lacking BIR2 (BIR1RING) bound to GRIM (Fig. 4A and D). Therefore the binding profile of Tn-IAP1 and its domains correlates with their ability to block HID- and GRIM-induced apoptosis (Fig. 3, C and D). This study indicates further that the BIR2 of Tn-IAP1 is essential for its anti-apoptotic activity against GRIM and HID.

In summary, we have demonstrated use of the apoptosis-inducing mutant of AcMNPV, vP35del, as a trap for cloning novel anti-cell death genes from an expression cDNA library derived from cultured insect cells. The screen used is simple...
and yet very powerful because of the nature of selection used. Combined with the selection is the visual production of OV that allows for independent confirmation of a productive infection. Also, because of homologous recombination and insertion of the foreign genes into the viral genome, those viruses that carry anti-cell death cDNA effectively amplify the gene from the initial pool of cDNAs. The deletion within the p35 gene adds to the stringency of the assay by preventing the production of revertant viruses and the expression of late viral genes that are required for the production of OV.

The use of this genetic screen led to the identification of a novel member of the IAP family of anti-apoptotic genes, Tn-IAP1, though cellular in origin, functions like its viral counterparts and blocks apoptosis induced by virus and other cell death inducers including HID, GRIM, and RPR. The high degree of similarity between Tn-IAP1 and the viral Cp-IAP and Op-IAP suggests a cellular origin for these viral IAPs and highlights the importance of acquired host genes in evolution of baculoviruses.

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