Apoptosis in Motion
AN APICAL, P35-INSSENSITIVE CASPASE MEDIATES PROGRAMMED CELL DEATH IN INSECT CELLS

Gulam A. Manji and Paul D. Friesen
From the Institute for Molecular Virology, and Department of Biochemistry, Graduate School and College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

Activation of caspases by proteolytic processing is a critical step during apoptosis in metazoans. Here we use high resolution time lapse microscopy to show a tight link between caspase activation and the morphological events delineating apoptosis in cultured SF21 cells from the moth Spodoptera frugiperda, a model insect system. The principal effector caspase, Sf-caspase-1, is proteolytically activated during SF21 apoptosis. To define the potential role of initiator caspases in vivo, we tested the effect of cell-permeable peptide inhibitors on pro-Sf-caspase-1 processing. Anti-caspase peptide analogues prevented apoptosis induced by diverse signals, including UV radiation and baculovirus infection. IETD-fmk potently inhibited the initial processing of pro-Sf-caspase-1 at the junction (TETD-G) of the large and small subunit, a cleavage that is blocked by inhibitor of apoptosis Op-IAP but not pancaspase inhibitor P35. Because Sf-caspase-1 was inhibited poorly by IETD-CHO, our data indicated that the protease responsible for the first step in pro-Sf-caspase-1 activation is a distinct apical caspase. Thus, Sf-caspase-1 activation is mediated by a novel, P35-resistant caspase. These findings support the hypothesis that apoptosis in insects, like that in mammals, involves a cascade of caspase activations.

Apoptosis is a dynamic process by which unwanted or diseased cells are disassembled in a rapid but systematic manner. Apoptotic cells undergo a series of dramatic and characteristic alterations in morphology that include chromatin condensation, DNA fragmentation, cytoskeletal reorganization, cell shrinkage, and membrane blebbing (1, 2). These irreversible changes in cellular architecture are initiated directly or indirectly by the proteolytic activity of the caspases, a highly conserved family of cysteiny1 aspartate-specific proteases that play a major role in programmed cell death (3–7). Not surprisingly, proper regulation of caspase activity is critical to apoptotic execution.

The caspases are activated from a latent proform (pro-caspase) by proteolytic excision of the large and small subunits that interact to generate the active enzyme. Pro-caspase processing occurs through proximity-induced autoactivation or by the activity of other proteases, including caspases (3, 4). In mammals, apoptotic signaling initiates a caspase cascade wherein activated initiator caspases proteolytically activate downstream effector caspases (6–8). Initiator caspases possess long prodomains that interact with diverse proteins that regulate protease activation. In contrast, effector caspases have short prodomains. Initiator and effector caspases often exhibit different substrate specificities in vitro (5, 9).

In invertebrates, programmed cell death plays a critical role in development, control of DNA damage, and defense of pathogens, including viruses (10–12). Caspases are required for apoptosis in insects, like that in mammals. However, the mechanisms by which caspases are activated and the hierarchy of apical and effector caspases are still unclear (10, 13, 14). On the basis of sequence similarity and biochemical activity, seven caspases have been identified in Drosophila melanogaster (Order Diptera) (15). Drosophila DRONC and DCP-2/DREDD possess long prodomains and by analogy to mammalian caspases are candidates as initiator caspases (16, 17). DCP-1, drICE, and DECAY contain short prodomains and therefore are likely effector caspases (18–20). Caspases have also been identified and characterized from lepidopteran insects (moths and butterflies). In particular, Sf-caspase-1 is the principal effector caspase of SF21 cells (21, 22), an established cell line from the nocturnal moth Spodoptera frugiperda (Order Lepidoptera). These invertebrate cells have been used extensively for studies on apoptosis because of their sensitivity to diverse death stimuli, including baculovirus infection, UV radiation, and overexpression of proapoptotic genes (i.e. Drosophila reaper, hid, and grim) and their response to known apoptotic regulators such as P35 and IAP (23–29).

Pro-Sf-caspase-1, which contains a short prodomain, is activated by sequential proteolytic cleavages that are initiated only upon apoptotic signaling. The first cleavage occurs between the large and small subunit at the caspase-recognition site TETD-G, which is also conserved in Drosophila DCP-1 and drICE. This initial cleavage event is blocked by baculovirus Op-IAP but is insensitive to the pancaspase inhibitor P35 (22). Op-IAP functions upstream from P35 to block apoptosis in Spodoptera (25, 30). Thus, it has been hypothesized that the first step in pro-Sf-caspase-1 activation is mediated by an apical caspase that is distinguished by its novel resistance to P35 (22).

To define the mechanism by which Sf-caspase-1 is activated upon apoptotic signaling and thereby gain insight into regulation of invertebrate effector caspases, we characterized the protease activity responsible for pro-Sf-caspase-1 activation. By using time lapse video microscopy of SF21 cells, we observed an exact correlation between the morphological hallmarks of
apoptosis and caspase activation. We report here that peptide-based fluoromethyl ketone inhibitors potently blocked SF21 apoptosis induced by multiple signals. In particular, zVAD-fmk and IETD-fmk prevented the initial proteolytic processing of pro-Sf-caspase-1 at TETD ↓ G. Because Sf-caspase-1 itself was inhibited poorly by IETD-CHO, our data indicated that the protease responsible for the first step in pro-Sf-caspase-1 activation is a distinct caspase, designated Sf-caspase-X. On the basis of these data, we concluded that the P35-insensitive activity of Sf-caspase-X is responsible for caspase activation in Spodoptera SF21 cells and that insects, like mammals, use a cascade of caspase-mediated events to execute apoptosis.

**EXPERIMENTAL PROCEDURES**

**Cells and Transfections**—S. frugiperda IPLB-SF21 (31) cells and Trichoplusia ni TN368 cells (32) were propagated in TC100 growth medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (HyClone Laboratories) and 2.6 mg of trypsin–broth/ml. SF21 cells were transfected as described previously (22). In brief, plasmid DNA in TC100 was mixed with N-1(2, 3-dioleoyloxy)propyl-N,N, N, trimethylammonium methyl sulfate liposomes for 30 min at ambient temperature. The transfection mixture was added to cell monolayers previously washed with TC100. After 4 h of gentle rocking, the transfection mixture was replaced with supplemented TC100. Transfection efficiencies ranged from 60 to 80% as judged by lacZ expression in control plates.

**UV Irradiation and Virus Infection**—SF21 cell monolayers were UV-B irradiated for 10 min at room temperature by using a Blak Lamp (UV, Upland, Calif) as described previously (33). For infection, cell monolayers were inoculated with extracellular budded virus at the indicated multiplicity of infection. Yields of infectious virus were measured by standard plaque assay using apoptosis-resistant TN368 cells. Wild-type L-1 AcMNPV (p35, iap) (34) and AcMNPV recombinants wt/lacZ (p35, iap) (35), v3p35 (p35, iap) and v3p35/lacZ (p35, iap) (36), and v0-p-IAP (p35, iap) (25) were described previously.

**Time Lapse Video Microscopy**—SF21 cells were plated onto glass coverslips mounted within 35-mm culture dishes. After cell attachment, growth medium was replaced, and the cells were UV irradiated or inoculated with virus as described. After a 2-h recovery period at 27°C, mineral oil was added to prevent evaporation. Cells were viewed on a Nikon (Tokyo, Japan) Diaphot microscope using a 100× oil immersion objective lens. Video images were obtained at the indicated intervals with a Photometrics Series 300 or Micromax digital camera. Images were background-subtracted and contrast-enhanced. QuickTime movies were produced using Adobe Premiere 5.1 using Cinepak compressor. Scale and time of compression are indicated.

**Quantitation of Apoptosis**—Levels of apoptosis induced in SF21 cell monolayers were determined by counting both apoptotic and viable, nonapoptotic cells using a Zeiss Axiosvert 135TV phase contrast microscope (magnification, 200×) equipped with a digital camera and IP Lab Spectrum P software. Cells undergoing plasma membrane blebbing and/or cell body fragmentation were scored as apoptosis; both hallmarkst were readily distinguished from viable cells (see Fig. 1). The mean ± standard deviation was calculated at the indicated times from the percentage of apoptotic cells of at least six evenly distributed fields of view and included from 1500 to 6000 cells.

**Treatment with Peptide Analogues**—Irreversible fluoromethyl ketone peptide inhibitors z-(benzoylcarbonyl)-DEVD-fmk, z-IETD-fmk, z-VAD-fmk, and z-ZFA-fmk (Calbiochem, San Diego) dissolved in Me_SO were mixed in supplemented TC100 and added to SF21 monolayers at the indicated concentrations. Cells were irradiated in the presence of peptide analogues and maintained at 27°C. During infection, peptide analogues were added 1 h after inoculation. Me_SO vehicle was used as control.

**Immunoblot Analysis**—Whole cell lysates or purified proteins were subjected to SDS-polyacrylamide gel electrophoresis and transferred to membranes. To detect Sf-caspase-1, immunoblots were incubated with a 2,000 dilution of α-Sfasp1 (22) and goat anti-rabbit immunoglobulin G (Pierce) conjugated to alkaline phosphatase. Color development was as described previously (36). Protein Purification—Escherichia coli strain BL21 (DE3) cells were induced with IPTG (isopropyl-β-D-thiogalactopyranoside) for overexpression from Sf-caspase-1-encoding pET plasmids (22). C-terminal His6-tagged proteins were purified by nickel (Ni2+) affinity chromatography as described previously (22, 24). Isolated proteins were >90% homoous as determined by SDS-polyacrylamide gel electrophoresis and Colloidal Burst Coomassie G Stain (Z axis). Protein concentrations were determined by using the Bio-Rad Protein Assay (Bio-Rad).

**Caspase Assays**—Sf-caspase-1 activity was measured in reactions (20 μl) containing 25 mM HEPES, pH 7.5, 1 mM EDTA, 0.1% CHAPS, 10% sucrose, 10 mM dithiothreitol, and 10 μM tetrapeptide substrates Ac-IETD-ambc or Ac-DEVD-ambc (Biomol Research). Accumulation of fluorometric product (amc) was monitored using a Molecular Dynamics Biolumin 960 Kinetic Fluorescence/Absorbance microplate reader (excitation, 360 nm; emission, 465 nm) at 30-s intervals for 30 min. Rate of product formation was obtained from the linear portion of the reaction curves within the first 10% of substrate depletion and averaged for triplicate assays. For inhibition assays, increasing concentrations of tetrapeptide thiolebodies Ac-IETD-CHO or Ac-DEVD-CHO (Biomol Research) were incubated with 200 fmol of purified Sf-caspase-1. After 1 h at ambient temperature, substrate Ac-DEVD-ambc (10 μM) was added, and residual caspase activity was measured as described above.

**Image Processing**—Stained gels and immunoblots were scanned at a resolution of 300 dots per inch by using a Hewlett Packard ScanJet lfxc. The resulting files were printed from Adobe Photoshop 3.0 and Illustrator 7.0 by using a Tektronics Phaser 450 dye sublimation printer.

**RESULTS**

**UV Radiation-induced Apoptosis of Cultured SF21 Cells: Time Lapse Microscopy**—Although many cellular components involved in apoptosis have been identified, little is known about the kinetics and morphological events of cell dismemberment. Cultured cells provide a unique view into the morphology of programmed cell death. In particular, SF21 cells from the moth S. frugiperda provide a useful model system for studies on both biochemical and morphological aspects of apoptosis (21, 23, 25, 27, 30, 33, 37–40). SF21 cells are especially attractive because of their sensitivity to diverse apoptotic stimuli and their classical apoptotic response, which includes degradation of chromosomal DNA into nucleosomal-sized fragments and vigorous membrane blebbing. Here, we used time lapse microscopy to document SF21 morphological events during apoptosis as a means to link them with intracellular biochemical processes.

SF21 cells rapidly succumb to UV radiation-induced apoptosis, which consumes >90% of a culture within 9 h (25, 33). Because of the large size (15–20 μm) and well defined nucleus of these cells, nuclear and cytoplasmic events during apoptosis were readily discerned by time lapse video microscopy (Fig. 1; see also Video 1 in supplementary material). The first sign of UV radiation-induced alterations was chromatin condensation, which included formation of multiple opaque or dense bodies (2–3 μm in diameter) within the nucleus (Fig. 1). The appearance of these spherical inclusions coincided with the early activation of cellular caspases 2–3 h after UV irradiation (33), which is consistent with caspase-mediated detachment of chromatin from the nuclear envelope and retraction into nuclear bodies (41–43). During this early period, other transformations were observed within the nucleus, including rapid migration of small particulate bodies and formation of vesicular-like structures. Immediately thereafter, apoptotic blebbing was uniformly initiated over the cell surface, upon which abundant microvilli-like structures were still observed. Membrane blebs first appeared as small rounded protrusions but grew rapidly to produce long extensions that ultimately detached from the main cell body to form apoptotic bodies. These vesicles were translucent or opaque, suggesting that their contents varied. Blebbing lasted for 30–45 min and consumed the cell, leaving behind a dense corpse. By 9–12 h after irradiation, only free floating apoptotic bodies and cell corpses remained (see Fig. 4A, panel ii).
that severely restricts virus yields in part because of premature host cell death (23, 26, 35, 44). Time lapse microscopy of SF21 cells inoculated with the \( p35 \) deletion mutant \( v_{\Delta p35} \) (Fig. 2; see also Video 2 in supplementary material) revealed that the morphological hallmarks of virus-induced apoptosis were similar to those induced by UV radiation. However, \( v_{\Delta p35} \)-induced apoptosis occurred later and was less synchronous. The first signs of apoptosis included formation of dense, refractile material within and around the inside edge of hypertrophied nuclei (Fig. 2, B and C). The appearance of this intranuclear density coincided with caspase activation, which begins 9 and 12 h after infection (22, 26). Because nuclear aggregates were absent in cells infected with wild-type virus that encodes caspase inhibitor P35 (see below), it is likely that this material is condensed chromatin resulting from caspase activity. Soon after these nuclear events, apoptotic blebbing was initiated. Blebbing initiated 9–19 h after infection (Fig. 2; see also Video 2 in supplementary material). This asynchrony may be due to variations in the timing of virus-induced apoptotic signaling. Blebbing was invariably followed by an unusual and striking series of fusions in which apoptotic bodies of an individual cell form a single spherical mass of vesicles. Although the mechanism for this resurrection-like process is unknown, it may involve virus-encoded surface proteins that mediate membrane fusion (45).

**Suppression of Apoptosis in AcMNPV \( p35^+ \)-infected Cells: Time Lapse Microscopy**—During infection, baculovirus P35 prevents premature host cell death by blocking apoptosis. The role that P35 plays in prolonging cell survival and contributing to virus productivity is dramatically illustrated by time lapse microscopy of wild-type AcMNPV-infected SF21 cells (Fig. 3; see also Video 3 in supplementary material). The first visible signs of infection occurred 9–12 h after inoculation, at which time the nucleus enlarged and the cell surface became ruffled. Extracellular budded virus, the first of two morphologically and temporally distinct forms of infectious virus (46), is shed in abundance at 9–20 h and probably accounts for the vesicular, nonapoptotic protrusions at the cell surface. During this period, host caspases are activated and subsequently inhibited by newly synthesized P35 (22, 26, 36). The second and largest virus particle, occluded virus (OV), appeared on the inside edge
of the hypertrophied nuclei beginning 22 h after infection (Fig. 3C). Composed of nucleocapsids embedded within a matrix of the protein polyhedrin (46), these polyhedral particles expanded to occupy the entire nucleus (Fig. 3D). The number, size (1–3 μm dia), and shape of OV particles varied between cells. OV are not produced unless apoptosis is blocked (23, 35). Finally, in a dynamic process that resembled necrosis, OV-containing cells expanded and ruptured (Fig. 3; see also Video 3 in supplementary material). Thus, as illustrated, the virus’ apparent strategy is to prolong cell survival long enough for progeny maturation, whereupon lysis facilitates virus dissemination.

Caspase-targeted Peptide Inhibitors Block SF21 Apoptosis—The morphological events delineating apoptosis in SF21 cells coincided with the early activation of caspases (21, 22, 26, 33). Current evidence suggests that multiple caspases participate in SF21 apoptosis. To define the in vivo role of initiator and effector caspases in this invertebrate system, we first tested the anti-apoptotic activity of peptide inhibitors that target the caspases, including the membrane permeable peptides DEVD-fmk and IETD-fmk. Both tetrapeptides were potent inhibitors of apoptosis induced by either UV radiation or baculovirus infection (Fig. 4). Incubation of UV irradiated cells with either tetrapeptide prevented all morphological signs of apoptosis, which was widespread in untreated cultures (Fig. 4A). Both peptides also blocked apoptosis induced by AcMNPV p35 deletion mutant vΔp35 (Fig. 4B). At the highest extracellular dose tested (200 μM), DEVD- and IETD-fmk reduced apoptosis to less than 5%. At lower concentrations (30–50 μM), IETD-fmk was two to three times more effective than DEVD-fmk. In contrast, the control fluoromethyl ketone FA-fmk failed to affect apoptosis induced by either death stimulus (data not shown). The intracellular concentration of each peptide inhibitor was not determined.

In addition to promoting cell survival, the caspase inhibitory peptides restored baculovirus multiplication. Upon treatment with DEVD- and IETD-fmk, vΔp35-infected SF21 cells accumulated OV particles at levels comparable with that of cells infected with wild-type virus that encodes caspase-inhibitor P35 (Fig. 5A). In the absence of tetrapeptide, OV was not produced. To compare the effects on infectious virus production, we monitored yields of budded virus from peptide-treated cells. DEVD-, IETD-, and zVAD-fmk increased virus yields 1,000-fold to levels that were comparable with p35+ viruses (Fig. 5B). Thus, the peptide inhibitors did not interfere with virus replication. The restoration of virus productivity ruled out the possibility that these inhibitors blocked virus-induced apoptosis by preventing virus replicative events that are required for apoptotic signaling (26).

Peptide Inhibitors Block Pro-Sf-caspase-1 Processing in Vivo—To identify the apoptotic step(s) affected by the permeable peptide inhibitors, we determined their effect on proteolytic activation of the SF21 effector caspase, Sf-caspase-1. Pro-Sf-caspase-1 is proteolytically activated in two steps starting with cleavage at the caspase-like recognition site TETD$^{195}$ G (Fig. 6A). The resulting p25 fragment is then cleaved at DEGD$^{28}$ A to remove the prodomain and generate the mature enzyme complete with large (p19) and small (p12) subunits (21, 22). Caspase processing was monitored by immunoblot analyses using α-SfCasp1 antiserum, which recognizes the proform, p25, and p19 subunit of Sf-caspase-1. Upon infection with p35 deletion mutant vΔp35, p19 was the predominant product (Fig. 6B, lane 2). DEVD-fmk decreased accumulation of p19 (lane 3). However, at equivalent concentrations, IETD-fmk and zVAD-fmk eliminated p19 and reduced p25 to background levels (compare lanes 4 and 5 with lane 1). As shown previously (22), P35 fails to block cleavage of pro-Sf-caspase-1 at TETD G but inhibits subsequent DXXD cleavages. Thus, p25 is the major processing intermediate upon infection with wild-type p35+ virus (lane 6). In the presence of DEVD-fmk, p25 and p19 accumulation was reduced (lane 7). However, in p35− virus-infected cells, IETD- and zVAD-fmk prevented the appearance of these intermediates (lanes 8 and 9). The effectiveness of IETD- and zVAD-fmk was comparable with that of Op-tap (lane 10), which fully blocks the initial cleavage of pro-Sf-caspase-1 (22, 30). Thus, IETD- and zVAD-fmk inhibited the protease responsible for the first TETD G activation cleavage of Sf-caspase-1 and indicated that this upstream activity is mediated by a caspase. Peptides IETD- and zVAD-fmk were more effective inhibitors of this caspase activity than DEVD-fmk, which blocked the downstream DXXD cleavages of pro-Sf-caspase-1.

Differential Inhibition of Sf-caspase-1 by IETD- and DEVD-CHO in Vitro—Caspases exhibit distinct selectivities for peptide substrates (9). The differential effects of IETD- and DEVD-fmk on in vivo processing of pro-Sf-caspase-1 and the
differences in P₄ to P₁ residues at each processing site (Fig. 6) suggested the participation of multiple caspases during activation. To assess the involvement of Sf-caspase-1 itself in this processing, we tested the in vitro sensitivity of purified Sf-caspase-1 to peptide aldehydes. As determined in dose response assays (Fig. 7), DEVD-CHO was ~100 times more effective than IETD-CHO for inhibiting Sf-caspase-1 (IC₅₀ = 1.8 and 180 nM, respectively). Human caspase-3, the mammalian counterpart to Sf-caspase-1 (21), also has a greater sensitivity to DEVD-CHO when compared with IETD-CHO (Kᵢ = 0.23 and 195 nM, respectively) (9). Consistent with these results, purified Sf-caspase-1 hydrolyzed DEVD-amc efficiently, whereas even high levels of protease cleaved IETD-amc poorly (data not shown). Thus, Sf-caspase-1 prefers DEVD over IETD-containing substrates. These data suggested that IETD-fmk inhibition of in vivo TETD-iG processing of pro-Sf-caspase-1 is principally due to inhibition of an apical caspase and not Sf-caspase-1 itself. This conclusion is consistent with the previous finding that in vivo processing of pro-Sf-caspase-1 at TETD-iG is insensitive to caspase inhibitor P35, which potently inhibits active Sf-caspase-1 (21, 22).
An Initiator Caspase in Insect Apoptosis

DISCUSSION

Role of an Apical Caspase in Apoptosis of Invertebrate SF21 Cells—Current evidence supports a cascade pathway for caspase activation during execution of apoptosis in the insect S. frugiperda (Fig. 8). The principal effector protease Sf-caspase-1 is activated by consecutive proteolytic steps, each mediated by a distinct enzyme. The first step involves cleavage of the large-small subunit junction (TETD G) by the apical caspase designated Sf-caspase-X. The second step removes the Sf-caspase-1 prodomain by a DXXD G cleavage to generate the mature enzyme. This maturation step is blocked in vivo by caspase inhibitor P35, whereas the initial cleavage step by Sf-caspase-X is not (22). Despite its resistance to P35 inhibition, Sf-caspase-X activity is fully inhibited in vivo by cell-permeable peptide analogues containing a P1-aspartate residue. Furthermore, Sf-caspase-X-mediated activation of Sf-caspase-1 is blocked directly or indirectly by baculovirus Op-IAP and probably its cellular homolog Sf-IAP (22, 47) (Fig. 8). The molecular mechanism by which Sf-caspase-X is activated by diverse apoptotic signals, including UV radiation and virus infection, remains to be determined.

Our data indicate that Sf-caspase-X is an apical caspase that is distinguished by its resistance to pancaspase inhibitor P35. Sf-caspase-X activity was inhibited in vivo by the peptide analogues zVAD-, IETD-, and less well by DEVD-fmk (Fig. 6). These inhibitors prevented cleavage of pro-Sf-caspase-1 at TETD G, an event required for commitment to apoptosis in SF21 cells. Cytosolic extracts of SF21 cells also contain a P35-insensitive Sf-caspase-X-like activity, which is inhibited by iodoacetate, IETD-CHO, and DEVD-CHO, but not FA-fmk or E64.2 Although resistant to P35 (22), Sf-caspase-X activity is blocked in vivo by baculovirus P49, a caspase-specific inhibitor that functions upstream from P35 and thus inhibits an apical caspase.3 Consistent with the involvement of an apical caspase in SF21 apoptosis, ectopic expression of a cDNA encoding human apical caspase-8 induced widespread apoptosis, whereas overexpression of effector caspases human caspase-3 and Sf-caspase-1 did not (data not shown and Refs. 22 and 30). Formal classification of Sf-caspase-X will require its purification and sequence determination.

Mechanisms of Caspase Activation in Insects—Our results that Sf-caspase-X is an apical caspase provide evidence that insects use a cascade of caspase-mediated cleavages to initiate apoptosis. In Drosophila, the pro-caspase forms of DCP-1, drICE, and DECAY contain short prodomains and undergo proteolytic activation at a TETD G site located at the large-small subunit junction analogous to that of pro-Sf-caspase-1 (22, 48). Thus, the function of Sf-caspase-X as an apical caspase is most likely conserved in Drosophila. Indeed, recent identification of large promdomain-containing caspasases, DREDD and DRONC, suggests that apical caspases exist in Drosophila (13, 16). DREDD and DRONC promdomains contain potential DED and CARD motifs that are found in human caspases-8 and -9, respectively, and participate in regulation of caspase activation (6). Interestingly, purified DRONC failed to cleave P35, nor was it affected by this caspase inhibitor (48, 49), suggesting that DRONC resembles P35-insensitive Sf-caspase-X. Additionally, p35 failed to block apoptosis induced by overexpression of dronc in transgenic flies (48, 49). These findings were unexpected because downstream effector caspases activated by a potential initiator caspase like DRONC should have been inhibited by pancaspase inhibitor P35, thereby preventing apoptosis. Subsequent studies have suggested that ectopic expression of dronc in Drosophila eyes can be blocked by p35 (50). Thus, additional experimentation is required to address the apical role of DRONC and DREDD in Drosophila.

Role of Apoptosis in Baculovirus Replication—Upon infection, diverse viruses induce apoptosis (51–53). In some cases, apoptosis facilitates virus multiplication and spread, as suggested by studies on influenza virus and Sindbis virus (53). Apoptosis can function as a host defense strategy that reduces virus yields and thereby prohibits virus spread. In particular, infection with apoptosis-causing baculovirus mutants reduces virus yields as much as 10,000-fold in SF21 cells (35). Moreover, the infectivity of these mutants in insect larvae is 25–1000-fold lower than that of wild-type apoptosis-suppressing virus, suggesting that apoptosis impedes baculovirus multiplication in the host (44).

Here, we demonstrated that caspase-specific peptide inhibitors blocked virus-induced apoptosis and restored multiplication of p35 and iap null mutants to wild-type levels (Figs. 4 and 5). These findings indicated that the primary function of baculovirus-encoded apoptotic inhibitors is to negate caspase activation or activity during infection and thereby block apoptosis. Moreover, there were no obvious negative effects of these caspase inhibitors on virus replication and maturation. Thus, host caspases are not required for any phase of baculovirus replication or infection. Collectively, these data argue that apoptosis is solely an anti-virus defense mechanism employed by the host. Thus, for certain viruses, suppression of the host suicide response provides a significant selective advantage. Understanding the molecular mechanisms by which such viruses circumvent this host defense strategy will continue to provide insight into the regulation of apoptosis.

Acknowledgments—We thank Alexander Verkhovsky, Thomas Keating, Vladimir Rodionov, and Gary Borisy (Laboratory of Molecular Biology, University of Wisconsin-Madison) for the use of time lapse microscopy instrumentation, assistance, and advice during this study. We thank Brooke Milde and Judit Jane-Valbuena for technical assistance and Brock Binkowski for helpful discussions.

2 B. Binkowski and P. Friesen, unpublished results.
3 S. Zoog, J. Schiller, J. Wetter, N. Chejanovsky, and P. Friesen, unpublished data.
REFERENCES

1. Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972) Brit. J. Cancer 26, 239–257
2. Mills, J. C., Stone, N. L., and Pittman, R. H. (1999) J. Cell Biol. 146, 703–708
3. Green, D. R. (1998) Cell 94, 695–698
4. Wolf, R. B., and Green, D. R. (1999) J. Biol. Chem. 274, 20049–20052
5. Nichols, D. W., and Thornberry, N. A. (1997) Trends Biochem. Sci. 22, 299–306
6. Thornberry, N. A., and Lazebnik, Y. (1998) Science 281, 1312–1316
7. Cryns, V., and Yuan, J. (1998) Genes Dev. 12, 1551–1570
8. Salvesen, G. S., and Dixit, V. M. (1997) Cell 91, 443–446
9. Garcia-Calvo, M., Peterson, E. P., Letting, B., Ruel, R., Nicholson, D. W., and Thornberry, N. A. (1998) J. Biol. Chem. 273, 32608–32615
10. Bergmann, A., Apagai, J., and Steller, H. (1998) Oncogene 17, 3215–3223
11. Clem, R. J., Hardwick, J. M., and Miller, L. K. (1996) Cell Death Differ. 3, 9–16
12. Nordstrom, J., and Abrams, J. M. (2000) Cell Death Differ. 7, 1035–1038
13. Abrams, J. M. (1999) Trends Cell Biol. 9, 435–440
14. Kumar, S., and Doumanis, J. (2000) Cell Death Differ. 7, 1039–1044
15. Vernoy, S. Y., Copeland, J., Ghosh, N., Griffin, E. E., Yoo, S. J., and Hay, B. A. (2000) J. Cell Biol. 150, 69–75
16. Dorstyn, L., Colussi, P. A., Quinn, L. M., Richardson, H., and Kumar, S. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 4307–4312
17. Chen, P., Rodriguez, A., Erskine, R., Thach, T., and Abrams, J. M. (1998) Dev. Biol. 201, 202–216
18. Song, Z. W., McCall, K., and Steller, H. (1997) Science 275, 536–540
19. Fraser, A. G., and Evan, G. I. (1997) EMBO J. 16, 2905–2913
20. Dorstyn, L., Read, S. H., Quinn, L. M., Richardson, H., and Kumar, S. (1999) J. Biol. Chem. 274, 30778–30783
21. Ahmad, M., Srivivasula, S. M., Wang, L. J., Litwack, G., Fernandez-Almemri, T., and Almemri, E. S. (1999) J. Biol. Chem. 274, 1421–1424
22. LaCount, D. J., Hang, S. F., Schneider, C. L., and Friesen, P. D. (2000) J. Biol. Chem. 275, 15657–15664
23. Clem, R. J., Fischheimer, M., and Miller, L. K. (1991) Science 254, 1388–1390
24. Bertin, J., Mandyk, S. M., LaCount, D. J., Gaur, S., Krebs, J. F., Armstrong, R. C., Tomasselli, K. J., and Friesen, P. D. (1996) J. Virol. 70, 6251–6259
25. Manji, G. A., Hozak, R. K., LaCount, D. J., and Friesen, P. D. (1997) J. Virol. 71, 4509–4516
26. LaCount, D. J., and Friesen, P. D. (1997) J. Virol. 71, 1530–1537
27. Vucic, D., Seshagiri, S., and Miller, L. K. (1997) Mol. Cell. Biol. 17, 667–676
28. Vucic, D., Kaiser, W. J., and Miller, L. K. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 10183–10188
29. Vucic, D., Kaiser, W. J., and Miller, L. K. (1998) Mol. Cell. Biol. 18, 3300–3309
30. Seshagiri, S., and Miller, L. K. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 13606–13611
31. Vaughn, J. L., Goodwin, R. H., Thompkins, G. L., and McCawley, P. (1977) In Vitro 13, 215–217
32. Hink, W. F. (1970) Nature 225, 466–467
33. Hozak, R. R., Manji, G. A., and Friesen, P. D. (2000) Mol. Cell. Biol. 20, 1877–1885
34. Lee, H. H., and Miller, L. K. (1978) J. Virol. 27, 754–767
35. Hersberger, P. A., Dickson, J. A., and Friesen, P. D. (1992) J. Virol. 66, 5525–5533
36. Hersberger, P. A., LaCount, D. J., and Friesen, P. D. (1994) J. Virol. 68, 3467–3477
37. Seshagiri, S., and Miller, L. K. (1997) Curr. Biol. 7, 455–460
38. Carter, J. L., Hersberger, P. A., and Friesen, P. D. (1994) J. Virol. 68, 7728–7737
39. Clem, R. J., and Miller, L. K. (1994) Mol. Cell. Biol. 14, 5212–5222
40. Jones, G., Jones, G., Zhou, L., Steller, H., and Chu, Y. (2000) J. Biol. Chem. 275, 22157–22165
41. Lazebnik, Y. A., Takahashi, A., Moir, R. D., Goldman, R. D., Poirier, G. G., Kaufmann, S. H., and Earnshaw, W. C. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 9042–9046
42. Rao, L., Perez, D., and White, E. (1996) J. Cell Biol. 135, 1441–1455
43. Orth, K., Chinnaiyan, A. M., Garm, M., Froliech, C. J., and Dixit, V. M. (1996) J. Biol. Chem. 271, 16443–16446
44. Clem, R. J., and Miller, L. K. (1993) J. Virol. 67, 3730–3738
45. Monoma, S. A., and Blissard, G. W. (1995) J. Virol. 69, 2583–2595
46. Friesen, P. D., and Miller, L. K. (2001) in Field’s Virology (Knipe, D. M., and Howley, P. M., eds) 4th Ed., Lippincott-Raven Publishers, Philadelphia, PA, in press
47. Huang, Q., Deveraux, Q. L., Maeda, S., Salvesen, G. S., Stennicke, H. R., Hammock, B. D., and Reed, J. C. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 1427–1432
48. Hawkins, C. J., Yoo, S. J., Peterson, E. P., Wang, S. L., Vernooy, S. Y., and Hay, B. A. (2000) J. Biol. Chem. 275, 27084–27093
49. Meier, M., Silke, J., Levers, S. J., and Evan, G. I. (2000) EMBO J. 19, 598–611
50. Quinn, L. M., Dorstyn, L., Mills, K., Colussi, P. A., Chen, P., Coombe, M., Abrams, J., Kumar, S., and Richardson, H. (2000) J. Biol. Chem. 275, 40416–40424
51. Shen, Y., and Shenk, T. E. (1995) Curr. Opin. Genet. Develop. 5, 105–111
52. Hardwick, J. M. (1997) Adv. Pharmacol. 41, 295–336
53. Roulston, A., Marcellus, R. C., and Branton, P. E. (1999) Annu. Rev. Micro. 53, 577–628
Apoptosis in Motion: AN APICAL, P35-INSENSITIVE CASPASE MEDIATES PROGRAMMED CELL DEATH IN INSECT CELLS
Gulam A. Manji and Paul D. Friesen

J. Biol. Chem. 2001, 276:16704-16710.
doi: 10.1074/jbc.M010179200 originally published online February 23, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010179200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2001/05/11/276.20.16704.DC1

This article cites 52 references, 35 of which can be accessed free at
http://www.jbc.org/content/276/20/16704.full.html#ref-list-1