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Fluorescent brightener inhibits apoptosis in baculovirus-infected gypsy moth larval midgut cells *in vitro*

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Abstract
Fluorescent brighteners significantly lower the LC_{50} and LT_{50} in a variety of nucleopolyhedrovirus–insect host systems. In larvae of the gypsy moth, *Lymantria dispar* (L.), a European NPV strain of virus (LdMNPV) does not normally replicate in the midgut, but addition of a fluorescent brightener (Calcofluor M2R) to the virus suspension results in productive infections. In the current study, we show that LdMNPV also does not replicate in a larval midgut primary cell culture system unless a fluorescent brightener (Blankophor P167) is added. Morphological and cellular changes characteristic of apoptotic cell death were noted in infected midgut cells *in vitro*. We used the TUNEL assay to measure apoptosis in virus-challenged midgut cell cultures at 24–48 h post-inoculation. A significant decrease in apoptotic midgut cells was noted in the presence of 0.01 M brightener. The inhibition of apoptosis and presumptive inhibition of shedding of infected midgut cells in the presence of fluorescent brightener in the insect midgut appeared to promote virus replication and are likely to be partly responsible for enhancement of LdMNPV activity that is observed in gypsy moth larvae.

Keywords: *Nucleopolyhedrovirus, fluorescent brightener, Blankophor P167, apoptosis,* Lymantria dispar*, cell cultures*

Introduction

Apoptosis, or programmed cell death, is caused by various factors including viral infection of numerous cell types. For example, herpes virus (Henderson et al. 1991), adenoviruses (White & Goodling 1994), and flaviviruses (Matsuda et al. 2005) have been shown to induce apoptosis in B cells, lymphocytes and hepatic cells, respectively. Apoptosis is characterized by cellular shrinkage, genomic degradation, granulation, chromatin condensation, and nuclear fragmentation coincident with blebbing of chromatin and cytoplasm from the cell surface. Induction of apoptosis is generally

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considered an important defense mechanism by cells against various pathogenic and toxic agents, including viral infection.

Baculoviruses also induce apoptosis in various insect cell lines (Clem et al. 1991). Virus-induced apoptosis has been described for *Autographa californica* nucleopolyhedrovirus (AcMNPV) and has served as a model system for the study of apoptotic molecular mechanisms during viral infection (Miller et al. 1998). *Spodoptera litura* (F.) cells infected with *Trichoplusia ni* (Hübner) multicapsid nucleopolyhedrovirus (TnMNPV) and *T. ni* cells infected with *Helicoverpa armigera* (Hübner) single capsid nuclear nucleopolyhedrovirus (HaSNPV) each undergo apoptosis, resulting in no production of progeny virus (Dai et al. 1999). However, the baculovirus AcMNPV carries the gene, *p35*, which encodes an inhibitor of activated caspases that blocks apoptosis. Baculoviruses also encode an additional class of apoptotic inhibitors called IAPs (Miller et al. 1998). In Sf-21 cells from *Spodoptera frugiperda* (J.E. Smith), P35 blocks apoptosis that is normally induced by viral infection or actinomycin-D (Clem & Miller 1993). Midgut cell lines from *Choristoneura fumiferana* (Clemens) also exhibited apoptosis after infection by AcMNPV (Palli et al. 1996). Whereas these previous studies generally utilized continuous cell lines whose actual cell types are typically unknown, we specifically studied midgut epithelial cells, the primary targets of occlusion-derived virus. Midgut cells are the first tissues to be infected by NPVs in the host insect and apoptotic events such as those described here and in previous studies may have a key role in the resistance of larvae to these pathogens.

Electron microscopic studies have suggested that the gypsy moth, *Lymantria dispar* (L.), nucleopolyhedrovirus (LdMNPV) does not replicate in the midgut (Shields 1985). However, in the presence of an optical brightener (e.g., Calcofluor M2R, a.k.a. Tinopal LPW), normal viral replication occurs (Adams et al. 1994). Studies by Washburn et al. (1998) have also shown that Calcofluor M2R enhanced the infectivity of AcMNPV in *T. ni* and *Heliothis virescens* (F.) larvae by blocking the sloughing of infected primary target cells in the midgut. Since apoptosis has been shown to occur under some conditions in continuous cell lines during the viral infection process, thereby eliminating virus before it can fully replicate, we examined the possibility that apoptosis of midgut cells may block the primary infection of gypsy moth larvae. Further, we tested the hypothesis that an optical brightener (Blankophor P167) rescues LdMNPV infections by blocking apoptosis.

Materials and methods

Insects and cells

L. dispar (New Jersey strain) was obtained from USDA-APHIS (Otis ANGB, MA). The insects were received as eggs and the larvae were reared on a wheat germ-based diet as described previously (Bell et al. 1981; Shapiro & Robertson 1992). Primary mixed midgut cell cultures were prepared according to the method described in (Sadrud-Din et al. 1996). Fourth stage larvae were surface-disinfected by immersion in 20% Septisol (Vetal Laboratories, St. Louis, MO), 0.1% *p*-hydroxybenzoic acid methyl ester (Sigma–Aldrich, St. Louis, MO) and 0.1% sodium hypochlorite for 2–3 min each. The midguts were removed from the larvae under sterile conditions within a laminar flow hood (Nuair, Plymouth, MN). The excised midguts were rinsed in a modified Ringer's solution containing 0.5% gentamicin and 0.01% antibiotic–antimycotic solution (Loeb & Hakim 1991). Small tissue pieces (3 mm^2) of midguts

were then rinsed with modified Grace's medium (GIBCO, Invitrogen, Carlsbad, CA) containing 7% fetal bovine serum, vitamin premix (Roche Chemicals, Nutley, NJ) and 1 ng 20-hydroxyecdysone/μl (Calbiochem Corp., La Jolla, CA). The midgut tissue samples were placed in 35-mm wells of six-well plates (Falcon, Becton-Dickinson, Oxnard, CA) in the same medium supplemented with fat body extract as described by Loeb and Hakim (1991) and Sadrud-Din et al. (1996). After 1 week at 4°C, the cells were sieved through 70-μm filters (Becton-Dickinson, Franklin Lakes, NJ). The sievate was washed by centrifugation (200 $\times g$, 2 min) with fresh media two times and then distributed among six-well plates. The culture medium was replaced weekly with fresh medium. Fat body extract was added to the culture medium (20 μL mL^{-1}) immediately before addition to the cultures. The cells were quantified from each well by taking counts of six independent fields of 1.0 mm^2. The viability of cells was determined by adding 0.01% trypan blue to the medium (Tolnai 1975). The cells were observed and photographed using a Nikon Diaphot-TMD inverted microscope and subsequently quantified by counting the proportion of cells excluding the dye.

Viruses

A clonal strain of wildtype LdMNPV, LDP-67 was produced and purified as described previously (McClintock et al. 1986). The LdMNPV-β_{GAL} recombinant virus containing the β-galactosidase gene under the control of the late polyhedrin promoter was provided by Dr James Slavicek (USFS, Delaware, OH). AcMNPV recombinant viruses used included AcMNPVP_{10}-lac Z (Williams et al. 1989), AcMNPV-HSP_{70}-lac Z (Engelhard et al. 1994), and AcMNPV_{GUS} (under the polyhedrin promoter, obtained as part of an expression vector system, Invitrogen, Carlsbad, CA). Virus inocula used to infect cells in culture were budded virus (BV) from cell cultures (IPLB-Ld652Y cells for gypsy moth virus and TN-368 cells for AcMNPV).

Experimental procedure

Cell treatments

Two-week-old midgut cultures were centrifuged at low speed (200 $\times g$, 2 min, room temperature). Aliquots of midgut cell precipitates ($\sim$20 000 cells) were centrifuged, washed in fresh medium and subsequently infected with LdMNPV-β_{GAL} BV alone or in the presence of Blankophor P167 (0.1–10 mM) (Bayer, Pittsburgh, PA). After 1 h of gentle shaking, the cells were washed twice by centrifugation and resuspended in fresh medium. The control groups of midgut cells were not inoculated with virus but were treated by the same rinse protocol. Cells were collected at various times (8–120 h) post-inoculation (pi) for staining with X-gal, X-glut or TUNEL analysis. Cells were fixed and stained using an X-gal kit (Invitrogen, Carlsbad, CA) for 2 h at 37°C at 1 mg mL^{-1} in PBS containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 2 mM magnesium chloride.

Apoptosis analysis

TUNEL. Each cell sample was washed in fresh Ringer's solution two or three times, resuspended in 200 μL of fresh Ringer's solution at room temperature and were

air-dried on 1% polylysine-coated slides overnight (10 μL, ~1000) cells). The slides containing cells were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). To detect the number of apoptotic cells, we used the terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay using an *in situ* cell death detection kit (AP; Roche Molecular Biochemicals, Indianapolis, IN). This technique detects single- and double-strand breaks in DNA, an indication of genome fragmentation that occurs during apoptosis. The cells were counterstained with 10% eosin y (Harleco, Philadelphia, PA) in 95% ethanol. Slides were mounted in Paramount®, photographs were taken using a digital camera (Polaroid Corp., Cambridge, MA) and images were captured (DMC Direct, Polaroid), enhanced (Photoshop, San Jose, CA) and digitally printed on photographic paper. The numbers of apoptotic (with blue-stained nuclei) and normal cells were counted from each slide; three replicates from each experiment were conducted, and six samples from each experiment were studied. The means of the percentage of apoptotic cells in each treatment were determined and both positive and negative controls were processed simultaneously. For the positive control, the slides of uninfected midgut cells were treated with DNAse (1 mg mL^{-1}) in 50 mM Tris–HCl, pH 7.5, 1 mg mL^{-1} bovine serum albumin (BSA) for 10 min at room temperature to induce DNA breaks prior to staining for apoptosis. For the negative controls, slides were incubated in the TUNEL solution without the terminal transferase (which is used to incorporate labeled nucleotides to DNA strand breaks).

Annexin V-Fluos staining

Membrane alterations in the apoptotic cells can be used to detect and quantify cells in apoptosis. During the apoptotic process, the cell loses membrane phospholipid asymmetry and exposes phosphatidylserine (PS) on the outer leaf of the plasma membrane. Annexin V, conjugated with fluorescein and propidium iodide, was used to differentiate viable and necrotic cells. We utilized an Annexin V-Fluos staining kit (Roche Molecular Biochemicals, Indianapolis, IN). Cells were washed in PBS, centrifuged at $100 \times g$, resuspended in a staining solution containing Annexin-V-FLUOS and propidium iodide, mounted on slides and examined with a Nikon E-600 epifluorescence microscope using an excitation wavelength of 450–500 nm and detection in the range of 515–565 nm. Cells were observed for cellular shrinkage, chromatin condensation, and blebbing of nuclei and cytoplasm at the cell surface using a light microscope ($\times 430$) and the numbers of apoptotic and normal cells of each type of midgut cell were determined. The resulting data were analyzed with Sigma Stat statistical software using ANOVA and Tukey's mean comparison.

Results

Preliminary observations with midgut cell cultures

Midgut cells obtained from late fourth stage gypsy moth larvae provided primary cultures in which three distinct cell types characteristic of the midgut epithelium could be distinguished morphologically as stem cells (55%), goblet cells (25%), and columnar epithelial cells (20%). Inoculation of these cultures with LdMNPV containing the β-galactosidase gene under the control of the late polyhedrin promoter

Table I. Effect of fluorescent brightener on the infection of midgut cells in culture by homologous and heterologous nucleopolyhedroviruses.

Virus	Treatment	Staining*
LdMNPV pol β_{GAL}	Untreated control	−
	Blankophor P167	+
AcMNPV $P_{10\text{-}LACZ}$	Untreated control	−
	Blankophor P167	−
$AcMNPV_{GUS}$	Untreated control	−
	Blankophor P167	−
AcMNPV- HSP_{70}-β_{GAL}	Untreated control	+
	Blankophor P167	+

*Positive response indicates the presence of blue color following the addition of substrate indicating replication of the respective virus. The same results were obtained when assayed at 24, 48, and 72 h post-inoculation of virus and with all three concentrations of brightener (0.1, 1.0, and 10.0 mM).

(LdMNPV-β_{GAL}), showed no evidence of replication[1] (Table I). Also shown in Table I, no evidence of virus replication was observed in midgut cultures inoculated with AcMNPV containing LacZ under the P_{10} gene or GUS under the polyhedrin gene (both of which are late promoters), although inoculation with AcMNPV containing a β_{GAL} gene under control of a *Drosophila* heat shock promoter did show gene activity. Alternatively, the addition of Blankophor P167 at concentrations of 0.1, 1.0, and 10.0 mM, allowed replication of LdMNPV at all three concentrations of brightener as indicated by the presence of β-galactosidase activity but similar treatment of AcMNPV-inoculated cultures with brightener had no effect on replication of these recombinant viruses. Further analysis of midgut cultures inoculated with the recombinant LdMNPV in the presence of brightener showed reporter gene activity (indicative of viral replication) and was observed in 60% of stem cells, 50% of columnar epithelial cells, and 50% of goblet cells at the highest concentration of brightener (Figure 1).

Rescue of LdMNPV β_{GAL} infection with fluorescent brightener

LdMNPV-β_{GAL} treated midgut cells were rinsed three times with cell culture medium at various times post inoculation (pi) and then stained with X-gal 48 h after addition of fluorescent brightener as described above. Treatment of midgut cultures with brightener at various times after inoculation with LdMNPV-β_{GAL} showed maximal rescue occurred when brightener was added 4–6 h pi (Figure 2) and was greatly diminished by 48 h pi. No virus activity (as represented by X-gal staining) could be detected in cultures treated with fluorescent brightener at 144 h.

NPV induction of apoptosis in L. dispar *midgut cells*

In uninfected midgut cells studied using the TUNEL method (Figure 3), apoptosis was observed in 12% of the cells. Among virus-infected midgut cells, the incidence

[1] In this paper, we are referring to replication as completing all three phases of the replication cycle ending with the production of progeny virions and a polyhedrin gene product.

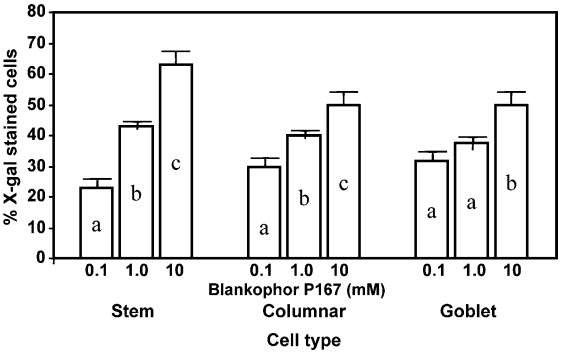

Figure 1. Susceptibility of midgut cells *in vitro* to infection with LdMNPV-β_{GAL} virus applied simultaneously with Blankophor P167 at concentrations of 0.1, 1.0, and 10.0 mM. At 48 h, the percentage of each cell type that was stained by X-gal for the presence of β-galactosidase was counted microscopically. Cells infected without fluorescent brightener showed no stained cells. Cells in six independent 1.0-mm^2 microscope fields were counted for each treatment. Marker bars are 1 standard deviation of three replicate tests and bars with the same letter were not significantly different ($p < 0.05$) as determined by ANOVA on the arc-sin transformation of the proportions within each cell type and Tukey's mean comparison test.

of apoptosis increased to 18% within 2 h and rose to approximately 40% after 24 h. If cultures were infected in the presence of fluorescent brightener, the incidence of apoptosis remained approximately 10% during the entire 120 h study (Figure 4).

Discussion

Since fluorescent brighteners were first identified as viral enhancers (e.g., reducing LC_{50} and LT_{50} values of insect viruses; Shapiro et al. 1992), these stilbenes have been investigated in several different insect virus systems (Vail et al. 1996; Zou & Young 1996; Li & Otvos 1999; Arakawa et al. 2000; Wang & Granados 2000). To date, several modes of action of these compounds involving different aspects of viral

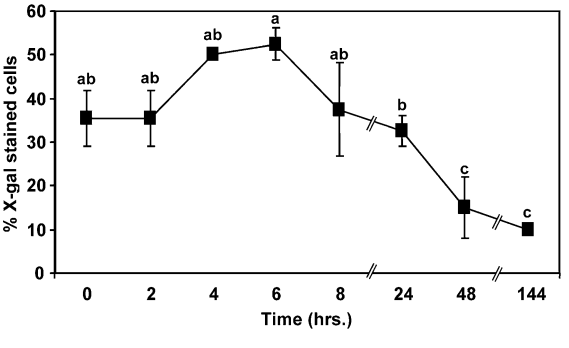

Figure 2. Susceptibility of midgut cells *in vitro* to infection with LdMNPV-β_{GAL} virus inoculated with Blankophor P167 following infection. Fluorescent brightener (1.0 mM) was added at various times post infection, stained with X-gal after 48 h and counted with a light microscope. Cells in six independent 1.0-mm^2 microscope fields were counted for each treatment. Control cells inoculated without fluorescent brightener at each time period showed no staining. Marker bars are one standard deviation of three replicate tests. Points labeled with the same letter are not significantly different when the arc-sin transformed data is analyzed by ANOVA and Tukey's mean comparison test.

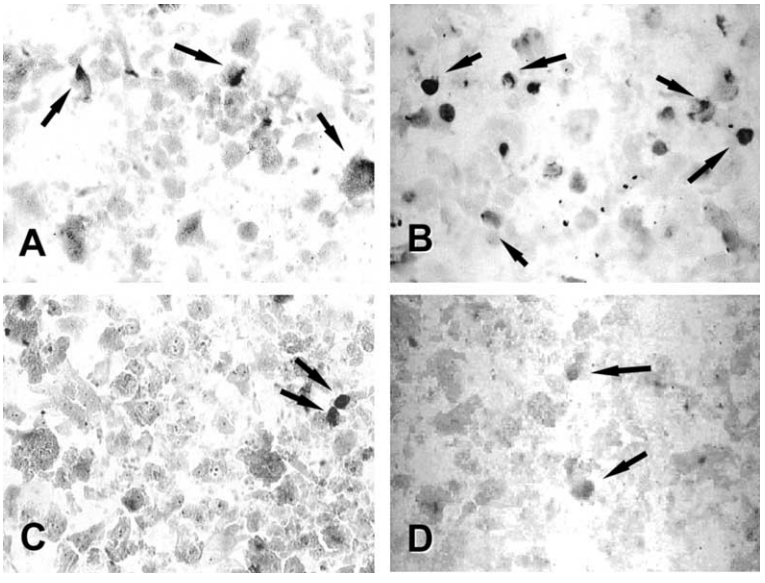

Figure 3. (A) Midgut cells of *L. dispar* after staining by TUNEL method exhibiting 10–15% of the apoptotic cells with nuclei stained blue (arrows); (B) LdMNPV infected midgut cells with about 40% of the cells apoptotic; (C) shows the cells after infecting with LdMNPV in the presence of 1% M2R, where the number of apoptotic cells are reduced to control levels; (D) positive control slide of midgut cells, which were treated with DNase to induce the DNA strand breaks.

pathogenesis have been documented (Adams et al. 1994; Sheppard & Shapiro 1994; Kirkpatrick et al. 1998; Hoover et al. 2000; Washburn et al. 2001). Stilbenes can act as UV protectants for insect viruses (Martignoni & Iwai 1985; Shapiro et al. 1992; Dougherty et al. 1996) as well as other entomopathogens, including fungi (Inglis et al.

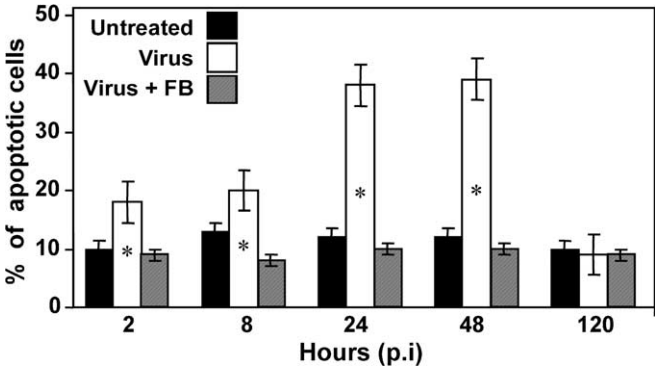

Figure 4. Effects of virus infection and fluorescent brightener on apoptosis of *L. dispar* midgut cells. Cells in culture were infected with wild-type LdMNPV with or without 0.01 M Blankophor P167. At various times post infection, the percentage of apoptotic cells were determined on samples analyzed using the TUNEL assay as described in the text. The reduced levels at 120 h are likely a result of all apoptotic cells having been cleared from the culture by this time. Black bars are untreated controls, white are virus-inoculated, and shaded are virus-inoculated plus fluorescent brightener. Each bar shows the mean and standard deviation (bars) of three replicate experiments. Bars marked with an asterisk (*) are significantly different from the controls as determined by ANOVA on the arc-sin transformation of the data and Tukey's mean comparison test.

1995) and nematodes (Nickle & Shapiro 1992, 1994) but these compounds also induce greater virus-caused mortality even without UV exposure, suggesting other modes of action may be functioning. Moreover, this improved efficacy only occurs when the brightener is present simultaneously with the virus in the insect midgut (Shapiro and Robertson 1992; Shapiro et al. 1992; Dougherty et al. 1995).

In light of this requirement, several studies have focused on the effects of fluorescent brighteners on the primary infection of the larval midgut. In ultrastructural studies of *L. dispar* larvae infected with LdMNPV with Calcofluor M2R, Shields and Podgwaite (1995) showed small holes occur in the peritrophic membrane (PM) at 0–24 h pi, followed by almost total loss of the membrane at later times. In some preliminary experiments using polyacrylamide gel electrophoresis on midguts dissected from larval infected with LdMNPV in the presence of Calcofluor M2R, we have observed only a few differences in the protein profiles of the PM at early times (0–8 h) but an almost complete absence of the PM by 96–120 h pi (Dougherty & Narang, unpublished data). Additionally, Wang and Granados (2000) have documented disruption of the cabbage looper PM by Calcofluor M2R alone. In their studies, PM proteins solubilized by Calcofluor M2R show strong chitin-binding affinity while intestinal mucin is degraded in Calcofluor-treated larvae. This disruption of the PM resulted in a retardation of larval development and increased their susceptibility to AcMNPV infection (Wang & Granados 2000), presumably by allowing virions greater access to midgut cells after destruction of the PM.

In addition to these PM effects, researchers have also seen other effects from fluorescent brighteners in virus infections. In AcMNPV-infected cabbage loopers and tobacco budworms, Washburn et al. (1998) observed that the sloughing of infected midgut cells was inhibited when Calcofluor M2R was added to the virus inocula. In our current study, we have extended these previous results by examining the effects of fluorescent brightener on baculovirus infection and pathogenesis on primary midgut cell cultures of *L. dispar* using both its homologous NPV (LdMNPV) and a heterologous NPV (AcMNPV).

Our preliminary experiments, as reported in Table I, verified the utility of primary midgut cell cultures as a method for studying the effects of fluorescent brighteners on the replication of baculoviruses. These tests showed that a recombinant homologous virus (LdMNPV-β_{GAL}) produced no staining (indicative of late gene activity) in midgut cells unless accompanied by a fluorescent brightener (Blankophor P167). Additionally, no late gene activity occurred when cells were inoculated with a heterologous virus (AcMNPV) with reporter genes under late promoters (lac Z under the P_{10} and GUS under the polyhedrin gene promoters) even with the presence of the brightener. However, staining did occur with this same virus containing lac Z under a constitutive reporter cassette, both with and without brightener, indicating the cells were capable of taking up and initiating the early stages of virus replication. Thus, the observations in our *in vitro* system are consistent with previously known activity of these viruses and fluorescent brightener since gypsy moth larvae are not susceptible *per os* to infection by AcMNPV and fluorescent brightener dramatically increases the pathogenicity of LdMNPV (Shapiro & Robertson 1992).

Additionally, as also seen *in vivo*, the brightener effects in midgut primary cultures are dose dependent, with each midgut cell type showing more infected cells (when inoculated with LdMNPV-β_{GAL} virus and stained with β-gal) with greater concentrations (Figure 1). The proportion of infected stem cells increased from 23 to 63% when

brightener concentrations were increased from 0.1 to 10 mM, while slightly lower increases were seen with columnar (30–50%) and goblet (32–50%) cells. This effect was observed even if brightener was added up to 48 h after the cells were inoculated with virus (Figure 2) with a maximal stimulation seen at 4 and 6 h pi. The concentrations at which the fluorescent brightener is effective in these cultures are also consistent with previous *in vivo* results, although, as one might expect, Blankophor P167 was effective at a slightly lower concentration *in vitro* (0.1 mM compared with 1.0 mM as the lowest effective concentration *in vivo*; Shapiro & Robertson 1992). Additionally, the evidence for late gene expression is consistent with the electron microscopic studies of Adams et al. (1994) in which virus replication is observed in the midgut of larvae fed LdMNPV only in combination with Calcofluor M2R brightener. Replication of LdMNPV in the midgut has only been observed when fluorescent brightener is fed concurrently with the virus (Dougherty et al. 1996), and since the PM was absent in our *in vitro* studies, the disruption or destruction of the PM by brightener (Wang & Granados 2000) apparently is not the sole mechanism for the observed change in infectivity of this tissue.

Palli et al. (1996) reported that differentiated midgut cell lines from the eastern spruce budworm *C. fumiferana* became apoptotic after infection with AcMNPV. The possibility that a similar effect was occurring in LdMNPV-infected midgut cells, and that fluorescent brightener was inhibiting apoptosis, would explain the observations of Adams et al. (1994), Washburn et al. (1998) and the results in our study. We have demonstrated that genomes and/or gene function could only be rescued for up to 48 h before cell death from apoptosis was irreversibly initiated. Fluorescent brightener apparently was able to block apoptosis effectively, which subsequently allowed LdMNPV replication in midgut cells. This hypothesis is consistent with data (Figure 2) in which the addition of fluorescent brightener had little success in 'rescuing' LdMNPV-β_{GAL} genome at times beyond 48 h.

Clem et al. (1991) first described the presence of apoptotic inhibitors in baculoviruses (for reviews, see Clem et al. 1996; Miller et al. 1998; Clarke & Clem 2003). Apoptosis and the ability to overcome anti-viral effects of apoptosis are important to the overall virulence of a virus. Whereas naturally occurring apoptotic suppressors such as P35 enhance the activities of NPVs, the numbers of suppressors present in any specific baculovirus and the tissues on which they exert their effects are not clear. While gene homologs for some apoptotic-suppressing genes (*iap-2* and *iap-3*) do exist in LdMNPV (Kuzio et al. 1999), their biological activity is currently unknown. Perhaps more interesting is the lack of homologs to two other apoptosis inhibitors, *p35* and *iap-1*. Recent evidence (Thiem & Chejanovsky 2004) indicates P35 can inhibit apoptosis in gypsy moth cells and one can hypothesize that its absence from the gypsy moth virus, LdMNPV, may be an important factor in the lack of replication being observed in the midgut of infected larvae.

In our LdMNPV-fluorescent brightener system, we have shown that the fluorescent brightener does suppress apoptosis that occurs when midgut cultures are inoculated with LdMNPV (Figures 3 and 4). Without brightener, about 40% of the midgut cells are in apoptosis by 24 h pi with LdMNPV while with brightener, the level remains equivalent to that seen in uninfected cells at approximately 10% (Figure 4). When the samples are examined 5 days pi, no difference is seen in the levels of apoptosis in all three treatments (control, virus, and virus plus brightener). This reduction of apoptosis in inoculated cultures at 5 days pi is consistent with *in vivo* effects of virus

exposure in which infected cells are sloughed (Washburn et al. 1998). In other words, the cultures may be recovered from the virus inoculation by this point in the culture so no further virus infections are occurring. Ultimately, we believe the inhibition of virus-induced apoptosis observed in these studies allows normal virus replication to proceed in these cultures and that this may also be a major mode of action of fluorescent brightener in infected insects.

In summary, several likely modes of action have been observed with fluorescent brighteners in virus–host systems. They have been shown to (1) act as UV protectants (Shapiro et al. 1992), (2) alter PM integrity (Sheppard & Shapiro 1994; Wang & Granados 2000), (3) inhibit sloughing of virus-infected midgut cells (Washburn et al. 1998; Hoover et al. 2000), and (4) suppress apoptosis as shown in this study, thereby allowing virus replication to occur in the midgut. Most likely, the dramatic improvement in infectivity of some viruses to host larvae is a result of a combination of these factors.

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