Changes in macromolecular synthesis of gypsy moth cell line IPLB-Ld652Y induced by Autographa californica nuclear polyhedrosis virus infection

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The aberrant replication of the Autographa californica multiple-enveloped nuclear polyhedrosis virus (AcMNPV) in the Lymantria dispar cell line IPLB-Ld652Y was used as a model system for the investigation of factors regulating baculovirus host specificity. A previous study of this system indicates that viral gene expression in infected cells is extremely attenuated and subsequently all cellular and viral protein synthesis is inhibited. In the present study, infection of IPLB-Ld652Y cells with AcMNPV photochemically inactivated in situ resulted in a rapid reduction in cell mitotic indices and cell growth, as well as inducing a series of distinct morphological changes in these cells. At the molecular level, infection with inactivated virus, followed by pulse labelling with [3H]thymidine, resulted in a rapid (0 to 2 h post-infection (p.i.)) and permanent inhibition of host cellular DNA synthesis. Assays of cellular DNA polymerases in isolated IPLB-Ld652Y nuclei confirmed the reduction in cellular DNA synthesis observed in intact cells and indicated an initial (0 to 2 h p.i.) reduction in the activity of aphidicolin-sensitive DNA polymerases. Activity of all cellular DNA polymerases was inhibited at later times p.i. Host cell protein synthesis was completely inhibited after 48 h p.i. Treatment of inactivated virus and virus-infected cells with various chemical and physical factors (i.e. pH and temperature) or lysosomotropic agents revealed that virus entry into cells and fusion of endocytic vesicles (containing virus) with lysosomes were essential for suppression of cellular macromolecular synthesis. The possible involvement of structural components of the AcMNPV virion in these effects is discussed.

Introduction

The nuclear polyhedrosis viruses (NPVs) have been shown to infect a variety of economically important lepidopteran insect species (Ignoffo, 1968; Gröner, 1986; Dougherty, 1987); however, many baculoviruses effectively replicate in and kill only one or a limited number of host species. Although some of the barriers to normal baculovirus replication in mammalian cell lines and cells of certain non-target insect species are defined (Carbonell et al., 1985; Carbonell & Miller, 1987), the factors which restrict baculovirus replication within the lepidoptera are poorly understood.

In our laboratory, we have been interested in identifying the molecular barriers which determine the host range of baculoviruses including the Autographa californica multiple-enveloped nuclear polyhedrosis virus (AcMNPV). An important aspect of this research has involved characterizing the abnormal or aborted AcMNPV replication in a cell line (IPLB-Ld652Y) derived from the gypsy moth, Lymantria dispar, which is not normally infected by the virus (McCIntock, 1986a). This cell line supports a limited degree of viral gene expression, followed by a total shutoff of host cellular and viral protein synthesis at approximately 20 h post-infection (p.i.). We report here that infection with AcMNPV, the DNA of which had been inactivated in situ, resulted in a permanent and irreversible inhibition of synthesis of macromolecules in IPLB-Ld652Y cells. In this cell line, therefore, normal viral replication may be inhibited by a viral component(s) which exerts an inhibitory effect on cell macromolecular synthesis. The
relevance of these data with regard to the factors regulating AcMNPV replication in the gypsy moth is discussed.

Methods

Cell lines and virus. The ovarian, pupa-derived L. dispar cell line IPLB-Ld652Y (Goodwin et al., 1977) was maintained in IPI-52B medium (Hazelton Research Products) as previously described (Goodwin & Adams, 1980). IPLB-LdElta cells (Lynn et al., 1988) were maintained in BML-TC/10 medium (Gardiner & Stockdale, 1975). Unless otherwise indicated, cells were incubated at 28 °C.

All infection experiments used a specific plaque-purified AcMNPV clone, 6R (McClimont, 1986b). Virus titres were determined by a modification of the endpoint dilution assay (Brown & Faulkner, 1975) in TN-368 cells (Hink, 1970).

Virus infections. Twelve-well plates (Linbro, Flow Laboratories) were inoculated with 2.5 x 10⁵ log phase IPLB-Ld652Y cells per well. After attachment, cells were inoculated with normal or inactivated AcMNPV (see Results; Inactivation of the AcMNPV genome) or non-occluded virus (NOV) at m.o.i. ranging from 0.5 to 100 TCID₅₀ per cell. Unless otherwise indicated, cells were infected at an m.o.i. of 50. After a 1 hour infection period, the virus inoculum was removed, the cells were rinsed three times with fresh tissue culture medium, and were incubated in the appropriate medium for the duration of the experiment.

For generation of AcMNPV from IPLB-LdElta cells, log phase cells were infected at an m.o.i. of 10. NOV was subsequently harvested 4 days p.i. and stored at −20 °C until use.

Inactivation of the viral genome. Short-wave u.v. or photochemical inactivation of viral genomes in situ, followed by infection of host cells with the inactivated virus, has been used previously to distinguish between cellular effects induced by virion components and those by viral gene products (Fenwick & Walker, 1978; Nishioka & Silverstein, 1978). Two different methods were utilized to inactivate the AcMNPV genome. The psoralen derivative, trioxsalen (4,5,8-trimethylpsoralen; Sigma) was used in combination with long-wave u.v. light (360 nm) to crosslink double-stranded nucleic acids in situ as previously described (Guo & Stoldt, 1985). AcMNPV nucleic acids were also inactivated by short-wave (254 nm) u.v. irradiation (Griego et al., 1985). Inactivation of the viral genome was confirmed by endpoint dilution assays on TN-368 cells.

Additionally, slot blots of total RNAs extracted from both psoralen/u.v.- or short-wave u.v.-inactivated virus-infected cells were probed with nick-translated, whole viral DNA or AcMNPV gene-specific probes (see below) to determine the level, if any, of virus-specific transcription.

Cell growth and mitotic indices. Cell growth rates were measured by initially plating 6.0 x 10⁵ IPLB-Ld652Y cells per well into 12-well plates. Cells were counted in a Neubauer haemocytometer with each data point representing the average of five separate counts. Mitotic cells were identified directly by phase microscopy or after staining with the vital stain hydroethidine (Polysciences) following the manufacturer’s instructions.

Tritiated thymidine incorporation. The effects of treatments on rates of cellular DNA synthesis were determined by pulse labelling with [³H]thymidine. Typical labelling experiments used IPI-52B or TNM-FH medium (Hink, 1970) with 5 μCi/ml of [³H]thymidine (NEN). After labelling, cells were lysed in 2% sodium sarcosinate and the nucleic acids precipitated with 5% trichloroacetic acid. The labelled DNA was then immobilized on Whatman glass fibre filters and the amount of incorporated [³H]thymidine was determined with a Searle Mark III Scintillation counter (Searle Analytical). Labelling experiments were repeated three times.

DNA synthesis in isolated nuclei. Characteristics of cellular DNA synthesis in isolated nuclei from uninfected, AcMNPV-infected and inactivated virus-infected IPLB-Ld652Y cells were examined. Nuclei from infected and uninfected cells were isolated by a previously described method (Wood et al., 1982). Conditions for optimum DNA synthesis in intact nuclei in vitro were as described by Flore et al. (1987). Activities of different DNA polymerases were determined using chemical inhibitors with demonstrated antagonistic effects against specific insect DNA polymerases (Miller, 1981; Sakaguchi & Boyd, 1985; Mikhailov et al., 1986; Wernette & Kaguni, 1986). Aphidicolin, an inhibitor of α and δ DNA polymerases (Kornberg, 1980), ddATP (inhibits primarily β and γ polymerases) and N-ethylmaleimide (inhibits α and γ polymerases) were all obtained from Sigma.

Isoptopic labelling of AcMNPV NOV. [³H]Thymidine-labelled AcMNPV NOV was produced in TN-368 cells. Three days p.i., the NOV was collected and purified as described previously (McClimont, 1986b). An aliquot of the virus sample (1/10th) was counted in a Searle Mark III Scintillation counter to determine the amount of incorporated radioactivity.

IPLB-Ld652Y cells were infected for 1 h with [³H]-labelled AcMNPV or [³H]-labelled inactivated virus at an m.o.i. of 10. After infection, cells were rinsed three times with sterile Locke's saline and refed with complete medium. Two h after infection cells were briefly treated with Pronase (10 min at 27 °C, Calbiochem) to remove any external virus, after which nucleic acids from whole cells or cell nuclei were precipitated with 5% trichloroacetic acid and immobilized on Whatman glass fibre filters. The amount of internalized virus was determined by dividing the c.p.m. of [³H] in whole infected cells by the total c.p.m. of [³H] in the original virus inoculum.

Infections in the presence of drugs. The effects of inhibitors and lysosomotropic agents on inactivated virus infection were determined by preincubation of the cells for 30 min with each drug followed by inactivated virus inoculation as described above. The virus inoculum and fresh medium controls also contained each drug at equivalent concentrations. All inhibitors and lysosomotropic agents were obtained from Sigma. Leupeptin, an inhibitor of certain acidic lysosomal proteases, was used at 1 mM; vinblastine, a microtubule poison which can inhibit fusion between phagocytic vacuoles and cellular lysosomes, was used at 0.1 mM. Propyleneamine and NH₄Cl₂, lysosomotropic weak bases, were used at concentrations of 25 mM and 20 mM, respectively.

The effects of drug treatments on cellular DNA synthesis in infected virus-infected IPLB-Ld652Y cells were determined by incubating infected and uninfected cells for 2 h p.i. in the presence of 5 μCi/ml [³H]thymidine. [³H]Thymidine incorporation into cellular DNA was determined as described above. Incorporation experiments in the presence of inhibitors were replicated three times. Potential virucidal effects associated with the lysosomotropic agents or inhibitors were assayed in TN-368 cells using AcMNPV exposed to each particular drug for 1 h before it was collected by centrifuging at 100000 g (2 h) and resuspended in fresh TNM-FH medium. In some experiments, inactivated virus was pretreated with polyclonal AcMNPV-specific or monoclonal anti-64K protein AcMNPV-specific antibodies (provided by Dr Loy Volkman, University of California, Berkeley, Ca., U.S.A.). Inactivation of normal AcMNPV by antibody treatment was confirmed by endpoint dilution assays on TN-368 cells.

Isolation of nucleic acids. AcMNPV DNA was extracted from purified occlusion bodies as previously described (McClimont et al., 1986a). AcMNPV-specific plasmids containing the IE-1 (Guarino & Summers, 1986) or IE-N (Carson et al., 1988) genes were generously
provided by Dr Linda Guarino, Texas A & M University, College Station, Tx., U.S.A. Total RNA fractions from uninfected, AcMNPV-infected and inactivated virus-infected IPLB-Ld652Y cells were extracted by the hot phenol–guanidinium hydrochloride method (Maniatis et al., 1982). Any contaminating DNA was removed by digestion for 1 h at 37 °C with RNase-free DNase (20 μg/ml; Fluka).

Preparation of RNA slot blots. Cellular RNA samples were denatured in 50% formamide and 10 x SSC for 15 min at 60 °C. Samples were immediately cooled on ice and applied to nitrocellulose filters in a Minifold II Slot blotter (Schleicher and Schuell). RNA filters were then air-dried (1 h) and baked at 80 °C for 2 h in a vacuum oven before hybridization.

Nick translation and hybridization conditions. Whole viral DNA or plasmids were labelled with [α-33P]CTP (NEN) by nick translation (Rigby et al., 1977) to specific activities of >1.0 × 10^6 c.p.m./μg. Immediately prior to hybridization, DNA probes were denatured by boiling (10 min) in 3 volumes of hybridization buffer (see below) and cooled on ice.

Nitrocellulose (or Nytran) filters were processed and hybridized with 32P-labelled viral DNA by previously described methods (Stoltz et al., 1986). Filters were air-dried and analysed by autoradiography.

Radiolabelling of cell proteins and SDS–PAGE. Log phase IPLB-Ld652Y cells were added to Linbro 12-Multiwell trays at a concentration of 2.5 × 10^5 cells/well and were inoculated with inactivated NOV at an m.o.i. of 50 TCID_{50} for 1 h. After infection, cells were rinsed twice and refed amino acid-free IPL-52B medium containing 40 μCi [35S]methionine (NEN) per ml. Cells were pulse-labelled for 4 h periods p.i. Radiolabelled cells were pelleted, suspended in disruption buffer (McClintock et al., 1986a) and boiled for 5 min. Cell samples were stored at −20 °C until use. Proteins were separated by SDS–PAGE (Laemmli, 1970). Staining and autoradiography techniques were as described previously (McClintock et al., 1986a).

Results

Inactivation of the AcMNPV genome

Inactivation of the AcMNPV genome by photochemical means was initially confirmed by endpoint dilution assays using TN-368 cells. Treating AcMNPV with trioxsalen plus long-wave u.v. or with short-wave u.v. light was equally effective in inactivating virus, each causing a 10^5-fold reduction in titre. The absence of AcMNPV-specific gene activity was confirmed by probing slot blots of total RNAs extracted from uninfected, AcMNPV-infected and inactivated virus-infected IPLB-Ld652Y cells with 32P-labelled whole AcMNPV DNA and AcMNPV gene-specific probes. Typical results are presented in Fig. 1. No detectable AcMNPV-specific gene activity was observed at any time p.i. with trioxsalen/u.v. or short-wave u.v.-inactivated virus. In all subsequent experiments, results obtained using trioxsalen/u.v.- and short-wave u.v.-inactivated virus were identical and both types of inactivated virus preparations will be referred to as i-v.

Actual entry of i-v into host cells was confirmed by infecting IPLB-Ld652Y cells with 3H-labelled

AcMNPV. Of the label present in the infectious AcMNPV inocula, 15-1% ± 2-3% (± s.d.) was internalized in host cells, and 9-1% ± 3-6% of the isotope present in the inactivated virus inocula was internalized. The amount of isotope internalized in cells as a result of virus inactivation was not statistically different (Student’s t-test, P ≤ 0-05).

Changes in cell morphology and growth rates

Cell morphology progressively changed after infection of IPLB-Ld652Y cells with i-v (Fig. 2). By 18 h p.i. some cellular clumping and blebbing was observed; formation of large dense cellular aggregates had occurred by 36 h p.i. The majority of cell aggregates subsequently (48 h p.i.) detached from the flask surface.

Infection with i-v also caused a permanent suppression
of cell growth and a reduction in mitotic indices (Fig. 3a and b). Despite the rapid suppression of cell growth, extensive cell mortality was not observed until 96 h p.i. (Fig. 3c). Most non-viable cells also retained their structural integrity until 7 to 10 days p.i.

**Protein synthesis inhibition**

Changes in protein synthesis in IPLB-Ld652Y cells infected with inactivated virus were monitored by pulse-labelling cellular proteins with \[^{35}S\]methionine (Fig. 4). Polypeptide profiles in uninfected and inactivated virus-infected IPLB-Ld652Y cells were alike until after 24 h p.i. when most cellular protein synthesis was inhibited in i-v-infected cells. At no time were AcMNPV infection-specific proteins observed in i-v-infected IPLB-Ld652Y cells.

To determine whether the lack of protein synthesis in i-v-infected cells was a consequence of changes in the ability of infected cells to internalize the radiolabelled methionine, uninfected and i-v-infected cells (at 72 and 96 h p.i.) were pulse-labelled with \[^{35}S\]methionine (40 μCi/ml) for 4 h, rinsed thoroughly and the amount of internalized radioisotope was counted. No significant difference in the amounts of internalized radioisotope between uninfected and i-v-infected cells was noted (data not shown).

**Inhibition of DNA synthesis**

Infection with i-v permanently inhibited IPLB-Ld652Y DNA synthesis in cell cultures (Fig. 5). Cellular DNA synthesis was immediately affected by infection, with incorporation of \[^{3}H\]thymidine reduced to 51.3% ±
6.7% of control levels by 0 to 2 h p.i. (Fig. 5b). Levels of incorporation of [3H]thymidine in infected cell cultures were reduced to approximately a third of normal by 12 h and to permanently below 10% of normal levels by 24 h p.i. (Fig. 5a).

In order to determine whether specific cellular DNA polymerases were selectively inhibited by i-v infection, cellular DNA synthesis in isolated IPLB-Ld652Y nuclei was assayed in the presence of various DNA polymerase inhibitors. Data presented in Table 1 indicate that the product synthesized was insensitive to RNase A but was sensitive to DNase I. Also, synthesis of DNA was inhibited by heat treatment of nuclei or by the presence of sodium pyrophosphate. DNA synthesis in uninfected cells was predominantly the product of aphidicolin-sensitive cellular DNA polymerases (i.e. α and/or δ polymerases). Incubation of nuclei with ddATP and N-ethylmaleimide indicated that the remaining DNA synthetic activity was primarily due to cellular β and γ DNA polymerases. The proportions of DNA synthetic activity attributed to the different types of DNA polymerases in normal uninfected IPLB-Ld652Y cells also agree closely with those previously observed in a variety of eukaryotic cells (Kornberg, 1980). At 2 h p.i. with i-v, cellular DNA synthesis was primarily aphidicolin-insensitive, thereby indicating that the majority of the DNA synthesized was the product of cellular β and γ DNA polymerases. DNA synthetic activity at 12 and 24 h p.i. also appeared to be primarily the product of cellular β and γ type polymerases. Levels of DNA synthesis in nuclei from AcMNPV-infected cells at 2 h p.i. were similar quantitatively and qualitatively to those in nuclei from i-v-infected cells. Further assays of DNA synthetic activity in normal AcMNPV-infected cells at 12 and 24 h p.i. were not done owing to the presence of virus-encoded DNA polymerase activity at these times p.i.

In order to study whether the i-v infection induced
Table 1. Effects of various treatments on DNA polymerase activity* in nuclei from uninfected, AcMNPV-infected or short-wave u.v.-inactivated AcMNPV (u.v./AcMNPV)-infected IPLB-Ld652Y cells

<table>
<thead>
<tr>
<th>Inhibitory treatment</th>
<th>AcMNPV Uninfected 2 h p.i.</th>
<th>AcMNPV u.v./AcMNPV 2 h p.i.</th>
<th>AcMNPV u.v./AcMNPV 12 h p.i.</th>
<th>AcMNPV u.v./AcMNPV 24 h p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>100% (341 790 c.p.m.)*</td>
<td>100% (101 622)</td>
<td>100% (88 950)</td>
<td>100% (39 960)</td>
</tr>
<tr>
<td>Heat (100 °C/5 min)</td>
<td>5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50 μg/ml DNase I</td>
<td>4%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50 μg/ml RNase A</td>
<td>94%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2% (w/v) sodium pyrophosphate</td>
<td>3%</td>
<td>24%</td>
<td>19%</td>
<td>16%</td>
</tr>
<tr>
<td>50 μM-ddATP</td>
<td>77%</td>
<td>29%</td>
<td>45%</td>
<td>30%</td>
</tr>
<tr>
<td>10 μM-N-ethylmaleimide</td>
<td>32%</td>
<td>26%</td>
<td>68%</td>
<td>66%</td>
</tr>
<tr>
<td>50 μg/ml aphidicolin</td>
<td>18%</td>
<td>79%</td>
<td>87%</td>
<td>87%</td>
</tr>
</tbody>
</table>

* Activity in all cases is expressed as the percentage of c.p.m. incorporated in nuclei from uninfected or virus-infected cells not subjected to any inhibitory treatment.

Table 2. Effects of chemical inhibitors and lysosomotropic weak bases on i-v infection-induced cellular DNA synthesis inhibition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Thymidine incorporation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>100 (11650)*</td>
</tr>
<tr>
<td>Inactivated AcMNPV (i-v)</td>
<td>59 ± 3.1</td>
</tr>
<tr>
<td>i-v/vinblastine</td>
<td>89 ± 3.6</td>
</tr>
<tr>
<td>i-v/leupeptin</td>
<td>104 ± 4.0</td>
</tr>
<tr>
<td>i-v/NH₄Cl₂</td>
<td>61 ± 1.5</td>
</tr>
<tr>
<td>i-v/propylamine</td>
<td>57 ± 2.5</td>
</tr>
</tbody>
</table>

* Activity is expressed as the percentage of c.p.m. (average ± standard deviation) in uninfected cells over a 2 h incorporation period.
† Virus was inactivated by short-wave u.v. irradiation as described in Methods.

Effects of various treatments on DNA and protein synthesis, as well as on host cell growth and morphology, were some function of the AcMNPV grown in TN-368 cells, AcMNPV NOV was grown in a second cell line (IPLB-LdEIta) permissive for AcMNPV replication. Virus from IPLB-LdEIta cells was inactivated photochemically and used to infect IPLB-Ld652Y cells. Changes in cell growth, morphology and macromolecular synthesis were identical to those induced with AcMNPV grown in TN-368 cells.

Fig. 5. Changes in [3H]thymidine incorporation within 120 h p.i. (a) and within the initial 12 h p.i. (b) in IPLB-Ld652Y cells as a result of infection by AcMNPV, trioxsalen/u.v.-inactivated (Ps Ac) and short-wave u.v.-inactivated AcMNPV (u.v. Ac). Error bars represent standard deviations for each data point.

Fig. 5. Changes in [3H]thymidine incorporation within 120 h p.i. (a) and within the initial 12 h p.i. (b) in IPLB-Ld652Y cells as a result of infection by AcMNPV, trioxsalen/u.v.-inactivated (Ps Ac) and short-wave u.v.-inactivated AcMNPV (u.v. Ac). Error bars represent standard deviations for each data point.

Effects of various treatments on the ability of i-v to alter IPLB-Ld652Y cell biology

To characterize the events involved in the i-v infection-induced suppression of host cellular macromolecular synthesis, virus inocula were treated with various physical or chemical agents prior to being used for infection.

Treatment of i-v with heat (56 °C, 20 min), acid pH (pH 3.0, 1 h), chloroform or neutralizing anti-64K protein monoclonal antibody eliminated the infection-induced effects on DNA and protein synthesis, cell morphology and cell growth. Incubation of i-v with non-neutralizing anti-64K protein monoclonal antibodies, however, had no effect on the capacity of i-v to induce changes in IPLB-Ld652Y cell biology. Overall, any treatment that inhibited virus entry into cells also eliminated the effects on cellular DNA and protein synthesis, cell morphology and cell growth rates.
Medium from which i-v was removed by centrifugation (150000 g for 3 h) had no discernible effect on IPLB-Ld652Y cells. The rapidity of DNA synthesis inhibition implied that an early event in the virus infection pathway, possibly involving the uptake and entry of virus particles into host cells, may interfere with host cellular DNA synthesis. Inhibitors that blocked fusion of lysosomes with phagocytic vesicles (vinblastine) or inhibited acidic proteases (leupeptin) abolished the ability of i-v to block cellular DNA synthesis (Table 2). Alternatively, i-v-infected cells incubated with lysosomotropic weak bases which prevent fusion between viral envelopes and the membranes of cellular phagolysosomes by raising the intralysosomal pH (NH4Cl2 and propylamine) exhibited rates of DNA synthesis not significantly different from those in i-v-infected cells in the absence of inhibitors. AcMNPV preincubated with any of the drugs used here was capable of initiating normal infections in TN-368 cells (data not shown).

Discussion

The barriers that limit the host range of baculoviruses within the lepidoptera can conceivably involve any aspect of viral attachment and entry, viral uncoating, DNA replication or expression of the viral genome. Although the barriers to normal replication in any particular baculovirus/non-permissive host combination are likely to be complex, a more thorough understanding of these factors may eventually permit the generation, by recombinant means, of baculoviruses with broadened host ranges.

In the present study we have expanded upon our previous investigation of the barriers to AcMNPV replication in IPLB-Ld652Y cells (McClintock et al., 1986a). In that study, AcMNPV virions were shown to be able to enter host cells, uncoat and initiate normal DNA replication. Expression of the viral genome is however extremely attenuated. Numerous cellular c.p.e.s are noted in infected cells; these include a cessation of cell growth, cell clumping and an abrupt and total inhibition of cellular protein synthesis by 20 h p.i. We demonstrate here that all of the c.p.e.s previously associated with AcMNPV infection in IPLB-Ld652Y cells could be induced by inactivated virions. The possibility that these effects are artefacts resulting from some membrane incompatibility between AcMNPV grown in a cell line from Trichoplusia ni (TN-368) and gypsy moth cells is considered unlikely as inactivated virus grown in IPLB-LdEITa cells exhibits similar activities in IPLB-Ld652Y cells. Also, similar activities associated with AcMNPV virions in another gypsy moth cell line (derived from the larval fat body tissue) have recently been demonstrated (E. M. Dougherty, unpublished observations).

The most rapid effect of inoculation with inactivated virus was a decline in cellular DNA synthesis, similar to that observed during normal baculovirus replication (Knudson & Tinsley, 1978) in which 12% of total DNA synthesis was cellular at 8 to 12 h p.i. This decline in DNA synthesis was evidenced by several lines of data, including a rapid reduction in cellular incorporation of [3H]thymidine in whole cells, a reduction in DNA synthesis by aphidicolin-sensitive DNA polymerase(s) in nuclei in vitro, and by a suppression of cell growth and mitosis. We therefore consider the DNA synthesis inhibition to be a significant effect induced by infection of IPLB-Ld652Y cells with inactivated AcMNPV. Indeed, we have been able to duplicate these effects artificially only through addition of high concentrations of potent DNA synthesis inhibitors (i.e. 20 to 40 μg/ml aphidicolin) to the tissue culture media.

The rapid reduction in cell DNA synthesis implied that some early step(s) in virus infection (i.e. adsorption, penetration or uncoating) was involved in inducing this effect. Previous research on early events in baculovirus infection indicates that the primary route of virus entry into cells is probably by adsorptive endocytosis and that subsequent uncoating of virions can be inhibited by lysosomotropic agents which interfere with low pH-dependent fusion of virus envelopes and phagolysosomal membranes (Volkman & Goldsmith, 1985). The elimination of the i-v infection-induced DNA synthesis inhibition effect by an inhibitor of fusion between lysosomes and phagocytic vesicles (vinblastine) and by an inhibitor of lysosomal acidic proteases (leupeptin), indicates that the fusion of cellular lysosomes with phagocytic vesicles (containing virus) is probably a critical step in the generation of the factor(s) which suppresses DNA synthesis. Also, since lysosomotropic weak bases did not affect i-v inhibition of DNA synthesis, the release of nucleocapsids into the cytoplasm may not be necessary for the cellular DNA synthesis inhibition. Possibly an AcMNPV virion component(s) was released or enzymatically modified by the action of phagolysosomal proteases on the virion nucleocapsid in the absence of normal viral uncoating. The mechanism(s) by which DNA synthesis is inhibited in i-v-infected cells is not completely understood, although it can be hypothesized that an antagonistic interaction between inactivated virions or virion components and cellular DNA polymerases may occur. Alternatively, the cellular DNA synthesis inhibition may be a secondary effect of some unidentified infection-induced toxic effect or some incompatibility between host cells and AcMNPV virions.

The most prominent feature of AcMNPV infection of IPLB-Ld652Y cells involves the total inhibition of...
cellular and viral protein synthesis by 20 h.p.i. (McClintock et al., 1986a). The current study suggests that some AcMNPV virion component(s) inhibits cellular protein synthesis in IPLB-Ld652Y cells. The longer time required for the total inhibition of protein synthesis in inactivated virus-infected cells (48 h compared to 20 h with normal AcMNPV) also implies that some AcMNPV-specific gene product modulates early protein synthesis inhibition. This is consistent with results found in herpes simplex virus infection of vertebrate cell lines in which cellular macromolecular synthesis inhibition is apparently regulated by both virion-associated factors and viral gene products (Sydiskis & Roizman, 1966; Honess & Roizman, 1973; Nishioka & Silverstein, 1978; Kwong et al., 1988). The types of interactions between AcMNPV virion components and cellular transcription/translation pathways are not known. Conceivably, some component(s) of the inactivated AcMNPV virion might interfere directly or indirectly with cellular protein synthesis by degrading host mRNAs or by binding to essential cellular factors involved in normal transcription/translation. Alternatively, the presence of inactivated virions in the cell may induce some cumulative injury to the host cell which could result in protein synthesis inhibition. Additionally, AcMNPV-specific protein synthesis in IPLB-Ld652Y cells has been demonstrated to be attenuated prior to total protein synthesis inhibition (McClintock et al., 1986a) and it is possible that virion components may also interfere with early viral protein synthesis in this cell line.

The mechanism(s) by which baculoviruses inhibit host cell macromolecular synthesis is not well understood. In this study, we have demonstrated that inactivated AcMNPV virions, in the absence of detectable viral gene expression, could induce a total shutoff of host cell protein synthesis and a significant reduction in cellular DNA synthesis. The data, while not conclusive, also suggest that a virion component(s) may mediate these effects. Obviously, the identification and characterization of any baculovirus virion factor(s) which inhibits host cell macromolecular synthesis could be a significant step in the development of compounds specifically designed to inhibit DNA and protein synthesis in insect cells.

References


Baculovirus virion component


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