Effect of an Autographa californica nuclear polyhedrosis virion component(s) on DNA synthesis and growth in several insect cell lines

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Summary. Thirteen different insect cell lines representing three different orders were infected with Autographa californica nuclear polyhedrosis virus (AcMNPV) whose genome had been inactivated in situ by photochemical means or by short wave UV irradiation. Changes in rates of cellular DNA synthesis, as measured by $[^3H]$thymidine incorporation, and cell growth were subsequently measured at various times post infection. Seven cell lines exhibited a significant decline in $[^3H]$thymidine incorporation (compared to control levels) during an initial 12 h period post infection, while three cell lines showed substantial declines in $[^3H]$thymidine incorporation over a 4 day period post infection. All cell lines which showed a significant decline in $[^3H]$thymidine over the duration of the experiment (4 days) also exhibited reduced cell growth rates. The role of a putative AcMNPV virion associated factor(s) in influencing these cellular events is discussed.

Introduction

The nuclear polyhedrosis viruses (NPVs) (family Baculoviridae, subgroup A) are large DNA viruses which infect insects. The pathways of infection as well as the cytopathic effects associated with baculovirus replication in vitro and in vivo are extensively documented and are reasonably well understood [1, 16, 37]. At the molecular level, baculovirus replication proceeds through the sequential expression of viral genes in a distinct cascade fashion [13, 25], culminating in the production of the two virion phenotypes characteristic of NPVs [35, 41]. Concomitant with viral macromolecular synthesis, normal host cellular DNA replication and protein synthesis are gradually inhibited [2, 25, 34]. However, it is not currently understood how host cell macromolecular synthesis is suppressed in infected cells.

In our laboratory, we have utilized a gypsy moth cell line (IPLB-Ld652Y)
which is semipermissive for replication of the *Autographa californica* multiple embedded NPV (AcMNPV) [24] to study the factors involved in regulating the host range of baculoviruses. Results of these studies indicate that a structural component(s) of the AcMNPV virion can elicit significantly reduced rates of host cell DNA synthesis after infection (Guzo et al., unpubl. obs.). The present study represents an expansion of these initial observations to other insect cell lines which are permissive, semipermissive, or non-permissive for AcMNPV replication. We report here that seven insect cell lines showed significant declines in cellular DNA synthesis within 12 h after infection with AcMNPV whose genome had been inactivated by photochemical means or by short wave UV irradiation. Furthermore, three cell lines (all normally permissive for AcMNPV) showed a sustained depression in DNA synthesis over a four day period post infection (p.i.). Declines in cellular DNA synthesis were also correlated with significantly reduced cell growth rates in some cell lines. We attribute these effects to a putative AcMNPV virion associated factor(s).

### Materials and methods

#### Cell lines and virus inoculations

Thirteen different insect cell lines were utilized in this study. The tissue origins of these cell lines, the media in which they were maintained, and their normal susceptibility to AcMNPV replication are listed in Table 1. For brevity, subsequent cell line designations throughout the text will omit the initials for laboratory of origin and will include only the species line designation.

All experiments utilized a plaque purified AcMNPV clone, designated 6R [25]. Non-occluded AcMNPV titers were determined using a modification of the end point dilution assay [3] in Tn 368 cells. Typical infection experiments were initiated by seeding Linbro twelve well plates (Linbro, Flow Lab, McLean, VA) with $1.0 \times 10^5$ log phase cells per well. After a twelve hour attachment period, cells were inoculated with normal AcMNPV non-occluded virus (NOV) or virus whose DNA was photochemically inactivated in situ (see below). Cells were inoculated for a 1 h period at a multiplicity of infection (m.o.i.) of 50 tissue culture infective doses at 50% infection (TCID$_{50}$) per cell. After inoculation, the virus inoculum was removed, the cells rinsed three times in sterile Lockes saline, and refed the appropriate media. To determine if any soluble factor(s) with biological activities was present in the virus inoculum, NOV was pelleted at 100,000 x g for 3 h, the supernatant removed and incubated with cells as per the virus inoculum.

Changes in cell numbers in inoculated and uninoculated cultures over time were determined utilizing a Neubauer hemocytometer. Cell growth rate experiments were independently replicated five times. Statistically significant differences ($P < 0.05$) between inoculated and control cell numbers were determined by pairwise Student's $t$ test at each time period. Additional analysis of the resultant curves was conducted by multiple comparisons based on least significant differences by Student's $t$ in a two way analysis of variance ($P < 0.05$). Cell viability was determined using the vital stain hydroethidine (Polysciences, Inc., Warrington, PA) as per the manufacturers' instructions.

#### Inactivation of the viral genome

AcMNPV DNA was photochemically inactivated in situ by a combination of long wave UV light (366 nm) and the psoralen derivative, trioxsalen (2,4,5-trimethylpsoralen) (Sigma