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Baculovirus Replication in a Mosquito (Dipteran) Cell Line

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The baculovirus from the lepidopteran host *Autographa californica* (alfalfa looper) was shown to replicate in a dipteran cell line without the production of characteristic polyhedral inclusion bodies. The low level of replication could not be detected by 50% tissue culture infective dose titrations, but was apparent by [^{3}H]thymidine labeling of the viral genome. Immunoprecipitation of the radioactive product confirmed baculovirus production.

Baculoviruses are large, rod-shaped deoxyribonucleic acid (DNA) insect viruses which have aroused considerable interest in recent years as biological control agents of insects. These viruses are reported to be very host specific and replicate in only one, or in a few, related host species (9). Typical infection in vivo and in vitro results in the production of proteinaceous crystals called polyhedral inclusion bodies (PIBs) into which a considerable proportion of virus particles become occluded. PIBs are therefore considered markers of virus replication. The baculoviruses are subdivided into two groups, nuclear polyhedrosis viruses (NPVs) and granulosis viruses, on the basis of the number of nucleocapsids embedded in each occlusion (11).

In this paper we provide evidence for the replication of *Autographa californica* (AC NPV) in the dipteran mosquito cell line (*Aedes aegypti*) without production of PIBs.

AC NPV is a baculovirus with a wide host range for lepidopterous insects, among which are *Trichoplusia ni* (cabbage looper) and *Spodoptera frugiperda* (fall armyworm). In laboratories, cell lines from these insects are used to grow AC NPV. The origin of the AC NPV employed in these studies has been previously described (4).

Goodwin et al. (1) reported that AC NPV failed to produce PIBs when tested against cell lines from several mosquitoes, including *Anopheles stephensii, Aedes albopictus,* and *Culex tritaeniorhynchus.* In our study, cells of *A. aegypti,* obtained from D. Roberts, Boyce Thompson Institute, Yonkers, N.Y., also failed to produce PIBs after exposure to AC NPV. Although no replication marker was produced, we continued further examination of the interaction between AC NPV and *A. aegypti* cells in culture.

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T. ni (2), *S. frugiperda* (10), and *A. aegypti* (8) cells were grown in Corning 25-cm^2 plastic T-flasks in TC-199 MK (3) supplemented with 10% fetal bovine serum. AC NPV cell cultures (inoculated and controls) were incubated at 28°C. Inoculum was prepared in the following manner: Supernatant fluids from AC NPV infected *S. frugiperda* cells (5 days postinoculation) were centrifuged at 320 × *g* for 15 min to remove floating cells. To further prevent introduction of permissive cells into a replication test, the centrifuged virus suspension was passed through a 0.45-μm Millex filter. Virus titrations were performed by the 50% tissue culture infective dose ($TCID_{50}$) method, using the Reed-Muench statistical analysis (6). Wells containing *T. ni* cells planted at 5 × 10^4 cells per ml were scored as positive if PIBs appeared after 5 days in inoculated cells. Typical titers for AC NPV produced in *S. frugiperda* cells were 10^7 $TCID_{50}$s per ml.

A. aegypti cells planted at 1 × 10^6 cells per ml and *T. ni* cells planted at 5 × 10^5 cells per ml were each exposed to 1 ml of an AC NPV suspension diluted to approximately 10^4 $TCID_{50}$s per ml. Supernatants from each flask were removed 6 days postinoculation and titrated as described above. Results were as follows: titers on days 0 and 6 were 4.39 and ~4 for *A. aegypti* and 4.39 and >7 for *T. ni,* respectively. As expected, the virus titer increased several log cycles in the permissive *T. ni* cell line. AC NPV in the mosquito cells showed no significant titer change. Although a titer increase was not observed, we considered the possibility of low-level viral replication. $TCID_{50}$ titrations proved to be insensitive in the detection of low-level replication of AC NPV in *A. aegypti* cells.

Since AC NPV is a DNA virus, thymidine is selectively incorporated into the viral genome during replication. Tritiated thymidine ([^{3}H]-thymidine, specific activity, 20 Ci/mmol; New England Nuclear Corp.) was added to TC-

199MK + 10% fetal bovine serum medium at a concentration of 50 μCi/ml. This medium was placed on cells which had been exposed to AC NPV (10^7 $TCID_{50}$s per ml) for 4 h and then washed to remove nonadsorbed virus particles. After 4 days, the supernatant was spun at 55,000 × *g* in a Beckman L3-50 centrifuge with a type 30 rotor for 1.5 h to pellet virus particles. The supernatant was removed and discarded, and the virus pellet was suspended in 0.2 ml of tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.4) overnight. The suspended virus pellet was then layered onto a 20 to 60% (wt/wt) linear sucrose gradient and spun at 80,000 × *g* in an SW41 rotor for 1 h. Fractions were collected by puncturing the bottom of the cellulose tube and collecting 0.2-ml quantities. Each fraction was spotted in 20-μl amounts onto Whatman glass fiber filter paper. Samples were placed in 10% trichloroacetic acid for 15 min at 4°C to precipitate macromolecules. Filters were then washed with 1 M HCl and 95% EtOH, dried, and placed in scintillation vials containing 3 ml of Yorktown liquid scintillation counting fluid. Each spotted fraction was counted for 1 min in the tritium window of an Intertechnique scintillation counter. Under these conditions, cellular oligo-nucleotides which may have picked up the [^{3}H]thymidine label are left at the very top of the gradient and are discarded. [^{3}H]thymidine-labeled virus migrates downward in the gradient and forms bands of radioactivity at characteristic positions in the gradient.

Figure 1 shows the results of an experiment in which *A. aegypti* cells were infected with AC NPV. Controls consisted of uninoculated *A. aegypti* cells processed by the identical procedure used for inoculated cultures. The peak occurring at a density of 1.24 g/cm^3 indicates incorporation of radioactive thymidine into DNA particles recovered from *A. aegypti* cells inoculated with AC NPV. No similar peak occurs in samples from control cultures. The peak was consistently found to occur in the same fractions as AC NPV produced in the permissive *T. ni* cell line. Thus, the physical characteristics of the particles were identical or very similar to the virus produced in permissive infection. Peak samples placed into wells containing *T. ni* cells caused production of an active baculovirus infection, as characterized by the appearance of PIBs.

Mosquito cell lines generally have 6 chromosomes, whereas lepidopteran cells usually have more than 60 (7). To check whether the cells we called *A. aegypti* were really mosquito cells, we did a chromosome spread and randomly counted the chromosomes of 100 cells. Chromosome numbers ranged from 3 to 9, with 76% of the cells having six chromosomes.

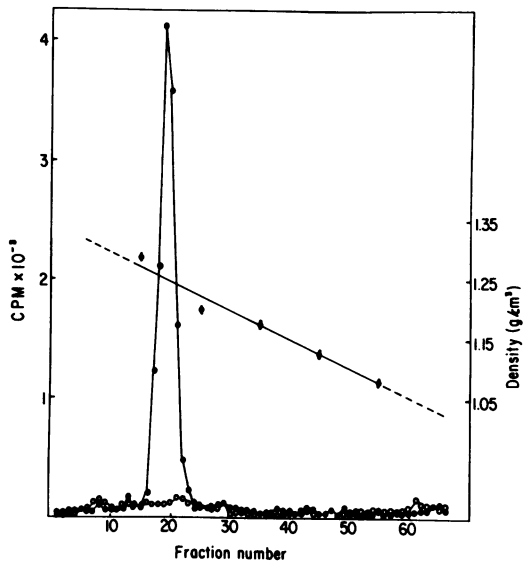

FIG. 1. *Incorporation of [^{3}H]thymidine into AC NPV in supernatant fluids from A. aegypti-infected cells. Challenge dose, 10^7 $TCID_{50}$s per ml; cell concentration, 1 × 10^6 cells per ml and 50 μCi of ^{3}H-thymidine per ml for 4 days at 28°C. Supernatant fluids from infected (●) and control (○) cells.*

All cell lines were also checked for mycoplasma contaminants by inoculation of cell supernatant fluids onto Difco PPLO agar. All were mycoplasma negative.

AC NPV antiserum produced in rabbits was added to 0.2 ml of the peak fractions from the gradient. Controls consisted of samples from the same gradient fractions to which Tris-hydrochloride was added instead of the immune serum. After suitable incubation (2 h at 35°C), goat anti-rabbit immunoglobulin G was added to precipitate rabbit globulin. If the latter contains AC NPV antibodies and if indeed AC NPV is the labeled entity, then it should be precipitated. Immune complexes were spun at 12,800 × *g* for 20 min and suspended in 0.2 ml of Tris-hydrochloride for scintillation counting as previously described. Results of this experiment were as follows: average precipitable counts per minute for the control (u_1) and the AC NPV antisera (u_2) were 39 and 262.8, respectively. Analysis by Student's *t* test shows that the control had significantly less counts in the precipitate than the test sample. This proves that the uptake of [^{3}H]thymidine was by AC NPV or a closely related virus and not by derepression of a latent deoxyribonucleic acid virus that migrates to the same place in the gradient as our sample virus.

The results of these experiments indicate that AC NPV is being produced in low amounts by a mosquito cell line. This is the first demonstration that baculoviruses may not be as host specific in vitro as was previously thought. In an earlier report, it was shown that a poikilothermic cell line was capable of synthesizing AC NPV antigens (5). In this experiment, AC NPV in vitro not only crossed species lines but also replicated in another order of insects. PIB production, which is generally considered the marker characteristic of the baculovirus, was absent in *A. aegypti* cells.

Since baculoviruses are finding increasing use as an alternative to chemical control methods, we believe that these results point out the need for a reevaluation of the host ranges and virus specificities of this important virus group.

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