Semipermissive Replication of a Nuclear Polyhedrosis Virus of *Autographa californica* in a Gypsy Moth Cell Line

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Several gypsy moth cell lines have been previously described as nonpermissive for the multiple-embedded nuclear polyhedrosis virus of *Autographa californica* (AcMNPV). In this report, we demonstrate the semipermissive infection of a gypsy moth cell line, IPLB-LD-652Y, with AcMNPV. IPLB-LD-652Y cells infected with AcMNPV produced classic cytopathic effects but failed to yield infectious progeny virus. Results of experiments employing DNA-DNA dot hybridization suggested that AcMNPV DNA synthesis was initiated from 8 to 12 h postinfection (p.i.), continued at a maximum rate from 12 to 20 h p.i., and declined from 20 to 36 h p.i. The rate of AcMNPV DNA synthesis approximated that observed in the permissive TN-368 cell line. AcMNPV-infected IPLB-LD-652Y cells, pulse-labeled with [³⁵S]methionine at various time intervals p.i. and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, revealed four virus-induced proteins, one novel to the semipermissive system and three early α proteins, synthesized from 1 to 20 h p.i. Thereafter, both host and viral protein synthesis was completely suppressed. These results suggest that AcMNPV adsorbed, penetrated, and initiated limited macromolecular synthesis in the semipermissive gypsy moth cell line. However, the infection cycle was restricted during the early phase of AcMNPV replication.

Nuclear polyhedrosis viruses (NPVs), classified in the family *Baculoviridae*, are structurally large and complex DNA-containing viruses infecting insects. The complex replicative cycles of the multiple-embedded NPV of *Autographa californica* (AcMNPV) and *Trichoplusia ni* have been described previously (5, 7, 20, 32). After viral DNA and protein synthesis, two forms of viral progeny are produced: nonoccluded virus (NOV), which bud through the plasma membrane (1, 28), and polyhedral inclusion bodies (PIBs), which contain nucleocapsids enveloped de novo (29) and occluded within the polyhedrin protein. Such studies, with different cell-virus systems for examining the mechanisms of viral replication, have provided a limited understanding of those factors that regulate the permissiveness of a cell for a particular virus.

Host cells infected with most DNA viruses can be categorized as permissive, semipermissive, or nonpermissive for viral replication. Infected permissive cells support complete DNA and protein synthesis, leading to the production of infectious progeny and lysis of the host cell. Semipermissive cells support limited viral replication. Nonpermissive cells survive viral challenge because if the virus enters the host, viral replication does not occur.

Early studies with simian virus 40 in nonpermissive cells suggested that abortive infections resulted from incomplete replication of the viral genome (13). In adenovirus systems, nonpermissiveness was characterized by the specific depression of late protein synthesis (9) and not by the inhibition of viral DNA replication (26). Previous investigations have described gypsy moth cell lines as nonpermissive for AcMNPV or the homologous NPV of *Lymantria dispar* (LdMNPV) (11; R. H. Goodwin, unpublished data). The criterion for nonpermissiveness was the inability of the cell line to exhibit cytopathic effects or produce occlusion bodies. Carpenter and Bilimoria (4) provided a limited description of the semipermissive replication of *Spodoptera*

frugiperda NPV (SfMNPV) in a homologous fall armyworm cell line based on kinetics, cytopathology, and morphogenesis of the infection. In this report we characterize the semipermissive infection of a gypsy moth cell line with AcMNPV, an infection cycle which is aborted during the early phase of viral replication.

MATERIALS AND METHODS

Cells and virus. The *L. dispar* cell subline IPLB-LD-652Y (12) was grown and maintained in KC-52B medium (KC Biologicals, Lenexa, Kans.) as previously described (10). The *T. ni* cell line TN-368 (14) was maintained in modified TNM · FH medium (16) containing 5% fetal calf serum.

A plaque-purified clone of AcMNPV, designated 6R, was obtained by three successive plaque isolations in TN-368 cells (15) and used in all experiments reported here. Unless otherwise noted, both cell lines were incubated at 27°C and observed for PIB formation by phase microscopy.

Endpoint dilution assay. Extracellular NOV production in AcMNPV-infected IPLB-LD-652Y cells was determined by the endpoint dilution assay (25) modified from those described earlier (3, 20). Corning 96-well microtiter trays were seeded with 10⁴ TN-368 cells per well, and experimental wells were inoculated with a serial 10-fold dilution of the virus and scored for PIB formation.

Plaque assay. Culture media from IPLB-LD-652Y cells infected with either active or UV-inactivated AcMNPV NOV were removed at various time intervals postinfection (p.i.), diluted serially, and assayed for NOV production in the TN-368 cell line by the plaque assay of Hink and Vail (17) and the endpoint dilution method described earlier. For UV inactivation, the viral inoculum was placed in a sterile shallow petri dish and exposed to short-wave UV light (UV Products, Inc., San Gabriel, Calif.) for 1 h with intermittent rocking.

Isotopic labeling and purification of AcMNPV NOV. Log-phase TN-368 cells were infected with AcMNPV NOV at a relatively low multiplicity of infection. After the removal of

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the virus inoculum, the cells were washed with sterile Locke solution, followed by the addition of complete medium containing 20 μ Ci of [35 S]methionine (Amersham Corp., Arlington Heights, Ill.) per ml. At 3 to 5 days p.i., the medium was collected, clarified of cells by centrifugation, and recentrifuged for 1 h at $20,000 \times g$. The pellet containing extracellular NOV was layered onto linear sucrose gradients (35 to 60% [wt/wt]) and centrifuged at $50,000 \times g$ for 2 h. The sucrose gradients were fractionated, and aliquots from each fraction were counted in a liquid scintillation spectrophotometer.

Preparation of samples for DNA-DNA dot hybridizations. DNA was extracted from uninfected and infected IPLB-LD-652Y cells by the method described by Meinkoth and Wahl (22). Briefly, log-phase IPLB-LD-652Y cells were adjusted to a final concentration of 4.5×10^5 cells per well and seeded into Linbro 12-multiwell trays. After attachment, cells were inoculated with AcMNPV NOV at a multiplicity of infection of 75 tissue culture infective doses at 50% infection ($TCID_{50}$) per cell. After adsorption, the virus inoculum was removed, and the cells were rinsed and incubated in complete medium. Samples were harvested, centrifuged at $95 \times g$ for 15 min, and suspended in 200 μ l of 10 mM Tris hydrochloride–1 mM EDTA (pH 7.4). An equal volume of 0.4 M Tris hydrochloride (pH 8.0)–100 mM EDTA–1% sodium dodecyl sulfate (SDS)–200 μ g of proteinase K per ml was added to the samples and incubated at 60°C for 1 h. The nucleic acids were extracted with buffered phenol, alkaline denatured, incubated at 60°C for 1 h, and neutralized with 2 M ammonium acetate (pH 7.0). For dot hybridizations, DNA samples were immobilized on nitrocellulose filters by the method described by Kafatos et al. (18) with the following modifications. With a Hybri Dot manifold (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), purified DNAs were applied to the nitrocellulose filters while under vacuum, followed by a 1 M ammonium acetate wash.

Isolation of viral DNA. AcMNPV DNA was isolated from purified PIBs by a modification of the procedure previously described by Miller and Dawes (23). Approximately 10^{10} PIBs per ml were dissolved with an equal volume of 0.1 M Na_2CO_3 at room temperature for 1 h and then stored at 4°C overnight. SDS, at a final concentration of 0.5%, was added to the alkali-dissolved solution and incubated at 60°C for 15 min. Viral DNA was extracted with buffered phenol and dialyzed extensively against TE buffer (10 mM Tris hydrochloride and 1 mM EDTA [pH 7.6]). The concentration of purified viral DNA was determined spectrophotometrically, and the DNA was characterized by agarose gel electrophoresis.

Nick translation and hybridization. Viral DNA was isotopically labeled by nick translation with [α - 32 P]dCTP (ICN Pharmaceuticals, Inc., Irvine, Calif.) by the procedure of Rigby et al. (27). The nick-translated viral DNA was extracted with phenol and separated from unincorporated label with a Sephadex G-50 column. The probe was denatured in 0.1 volume of 1 M NaOH, boiled for 5 min, and neutralized with 0.1 volume of 1 N HCl.

Just prior to hybridization, the nitrocellulose filters containing the denatured DNA samples were prehybridized with $10\times$ Denhardt solution ($1\times$ Denhardt solution is 0.01% Ficoll [Pharmacia Fine Chemicals, Piscataway, N.J.], 0.01% polyvinylpyrrolidone, and 0.01% bovine serum albumin [Sigma Chemical Co., St. Louis, Mo.]), $4\times$ SET buffer ($1\times$ SET is 0.15 M NaCl, 0.03 M Tris hydrochloride [pH 8.0], and 1 mM EDTA), 0.2% SDS, 150 μ g of tRNA (previously extracted with TE-saturated phenol-chloroform [1:1,

vol/vol] and precipitated with ethanol) per ml, and 50% deionized formamide at 37°C for 24 h. After prehybridization, the 32 P-labeled viral DNA probe (500,000 cpm/ml) was boiled for 5 min, placed into the Seal-a-Bag (Dazey Corp.) containing the hybridization mixture, and incubated at 37°C for 48 h. After hybridization, the filters were rinsed five times at 55°C for 10 min each with $1\times$ buffer containing 2.75 M NaCl, 20 mM Tris-Base, 0.01 N HCl, and 0.1% SDS, followed by five 10-min rinses in $0.1\times$ buffer. The filters were dried and processed for autoradiography. After autoradiography, the sample DNA dots from uninfected and infected cell preparations were excised and analyzed by liquid scintillation spectrophotometry.

Radiolabeling of infected-cell proteins. Linbro 12-multiwell trays were seeded with 4.5×10^5 IPLB-LD-652Y or TN-368 cells per well and infected with AcMNPV NOV at a multiplicity of infection of 75 and 10 $TCID_{50}$ per cell, respectively, for 1 h with intermittent rocking. After adsorption, the cells were rinsed and refed with either complete medium or amino acid-free medium containing 20 μ Ci of [35 S]methionine per ml. Pulse-labeled cell pellets were suspended in disruption buffer (2% [wt/vol] SDS, 2% 2-mercaptoethanol, 20% [wt/vol] sucrose, and 0.05% [wt/vol] bromophenol blue in 5 mM Tris hydrochloride [pH 6.8]) and boiled for 3 to 5 min. The solubilized cells were stored at –20°C until they were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

SDS-PAGE. Proteins were separated and analyzed by SDS-PAGE with the buffer system described by Laemmli (21). After electrophoresis, the gels were stained with Coomassie brilliant blue, destained in 10% glacial acetic acid and 30% methanol, dried under vacuum on Whatman no. 1 filter paper, and exposed to Kodak X-ray film. The molecular weights of the viral-induced proteins were determined by comparing their electrophoretic mobilities to those of standard molecular weight markers (Bethesda Research Laboratories) and [35 S]methionine-labeled AcMNPV proteins synthesized in TN-368 cells. The following molecular weight standards were used: phosphorylase *b* (97,400), bovine serum albumin (68,000), ovalbumin (43,000), α chymotrypsinogen (25,700), β -lactoglobulin (18,400), and lysozyme (14,300).

RESULTS

Cytopathic effects in AcMNPV-infected gypsy moth cells. IPLB-LD-652Y, a homologous and permissive cell line for LdMNPV, was previously described as a nonpermissive cell line for AcMNPV. To understand some of the mechanisms involved in regulation of viral infection by host cells, IPLB-LD-652Y cells were challenged with AcMNPV. IPLB-LD-652Y cells infected with AcMNPV or UV-inactivated AcMNPV NOV were monitored by phase microscopy at various intervals for change in overall morphology. Infection of IPLB-LD-652Y cells with AcMNPV produced subtle but reproducible cytopathic effects after several days of growth. No cytopathic effects were detected between 0 and 24 h p.i. (Fig. 1a); however, AcMNPV-infected IPLB-LD-652Y cells did exhibit altered morphologies at 24 h p.i. Some cells displayed two to three fibroblastic extensions typical of logarithmic-phase IPLB-LD-652Y cells, while spherical cells tended to form aggregates (Fig. 1a).

At 48 h p.i., the infected cell population formed aggregates and exhibited spherical morphology, and the vast majority of cells were granular in nature (Fig. 1b). At 96 h p.i., the infected cells detached, aggregated (Fig. 1c), and exhibited

FIG. 1. Phase-contrast microscopy of the IPLB-LD-652Y cell line infected with AcMNPV. AcMNPV-infected IPLB-LD-652Y cells at 24 (a), 48 (b), and 96 (c) h p.i. IPLB-LD-652Y cells infected with UV-inactivated AcMNPV NOV (as described in Materials and Methods) exhibited morphologies typical of normal gypsy moth cells at 24 h p.i. (d).

decreased viability as assessed by the trypan blue exclusion test. PIBs were not observed at any time during the replicative cycle. IPLB-LD-652Y cells infected with UV-inactivated AcMNPV NOV were morphologically typical of normal cells at 24 (Fig. 1d), 48, and up to 96 h p.i.

Absence of NOV production in AcMNPV-infected IPLB-LD-652Y cells. NOV production and infectivity in AcMNPV-infected IPLB-LD-652Y cells was assessed by harvesting culture media from infected cells at 6, 24, 48, and 96 h p.i. Results of such experiments with the TN-368 cell line in the endpoint dilution and plaque assays suggested that AcMNPV NOV production did not occur in the IPLB-LD-652Y cell line. The NOV titer at 6 h p.i. was attributed to the virus inoculum, which remained constant in samples collected at 24, 48, and 96 h p.i. (Table 1).

AcMNPV NOV production in IPLB-LD-652Y cells was also assessed in culture media from infected cells radiola-

beled for 3 days p.i. with [35 S]methionine with density gradients. Controls consisted of AcMNPV-infected TN-368 cells that were processed in the same fashion as AcMNPV-infected gypsy moth cells. No radioactivity was detected in the gradient containing the sample from AcMNPV-infected IPLB-LD-652Y cells, whereas the gradient containing the pellet from AcMNPV-infected TN-368 cells contained a single band, with the greatest amount of radioactivity in the area of the gradient in which AcMNPV NOV was shown to band (Fig. 2). From these results, we concluded that AcMNPV did not produce NOV in the IPLB-LD-652Y cell line.

AcMNPV DNA replication in IPLB-LD-652Y cells. Since AcMNPV-infected gypsy moth cells displayed cytopathic effects, we determined whether DNA replication occurred in this nonpermissive, heterologous cell line. Viral DNA synthesis of AcMNPV-infected IPLB-LD-652Y cells was mon-

TABLE 1. Determination of production and infectivity of AcMNPV NOV from AcMNPV-infected IPLB-LD-652Y cells

Time (h p.i.)	Endpoint dilution (TCID ₅₀ /ml) in TN-368 cells		Plaque assay (PFU/ml) in TN-368 cells	
	Active ^a	UV inactivated	Active	UV inactivated
6	3.25×10^4	1.68×10^2	1.65×10^4	2.00×10^1
24	2.75×10^4	2.55×10^2	1.80×10^4	2.00×10^1
48	2.85×10^4	2.18×10^2	2.40×10^4	2.00×10^1
96	2.92×10^4	4.18×10^1	1.80×10^4	2.00×10^0

^a The initial titer of virus stock was 3.98×10^7 TCID₅₀ per ml.

itored by DNA-DNA dot hybridizations. Using ³²P-labeled AcMNPV DNA to probe DNA from AcMNPV-infected cells, we detected only small amounts of intracellular AcMNPV DNA between 4 and 8 h p.i. (Fig. 3). These hybridization responses represented the parental genome from the virus inoculum. From 8 to 12 h p.i., total intensity of the viral DNA-DNA dots increased as measured by autoradiography and liquid scintillation counting. We concluded from these data that AcMNPV DNA synthesis in IPLB-LD-652Y cells was initiated from 8 to 12 h p.i., continued at a maximum rate from 12 to 20 h p.i., and ceased from 20 to 36 h p.i.

Protein synthesis in AcMNPV-infected TN-368 and IPLB-LD-652Y cells. As a comparative control for the semipermissive experiments and to establish a basic course of infected-cell-specific protein expression in AcMNPV-infected TN-368 cells, the synthesis of viral-induced proteins was monitored by pulse-labeling infected cells at various times p.i. with [³⁵S]methionine. Figure 4 shows an autoradiogram of labeled proteins from AcMNPV-infected TN-368 cells during the first 20 h p.i. and two additional samples collected from 34 to 36 h and from 46 to 48 h p.i. From these data, we divided AcMNPV-directed protein synthesis into sequential replicative phases. During the early (α) phase of replication, four viral-induced proteins with molecular weights of 46,500, 35,000, 34,000, and 31,000 were detected from 2 to 8 h p.i. During the second, intermediate (β) phase of protein synthesis, between 8 and 12 h p.i., a total of nine proteins with apparent molecular weights of 67,500, 45,000, 41,000, 39,000, 27,000, 24,000, 22,000, 21,000, and 16,500 were detected by SDS-PAGE. A total of three additional viral-induced proteins were detected during the last (γ) replicative phase. One protein, first detected from 12 to 14 h p.i., had a molecular weight of 33,000, similar to that of the polyhedrin protein. The other two proteins, with molecular weights of 15,700 and 15,000, were detected at 16 h p.i. No additional viral-induced proteins were detected after 16 h p.i. These results, with our plaque isolate of AcMNPV (6R), were essentially in agreement with those of Wood (31), showing

FIG. 2. Sucrose density gradients of AcMNPV nonoccluded virus. Culture media were harvested from AcMNPV-infected TN-368 cells and AcMNPV-infected IPLB-LD-652Y cells radiolabeled with [³⁵S]methionine. Samples were purified from infected cells, centrifuged on a linear sucrose gradient, fractionated, and counted on a spectrophotometer. Symbols: ●—●, [³⁵S]methionine-labeled NOV from AcMNPV-infected TN-368 cells; ○—○, culture samples from AcMNPV-infected IPLB-LD-652Y cells radiolabeled with [³⁵S]methionine; —, density.

FIG. 3. Kinetics of viral DNA synthesis in AcMNPV-infected IPLB-LD-652Y cells. Viral DNA synthesis was followed by DNA-DNA dot hybridization of nick-translated AcMNPV DNA to total DNA extracted from AcMNPV-infected IPLB-LD-652Y cells (4.5×10^5 cells per $200 \mu\text{l}$) at various time intervals p.i. DNA from uninfected control cells was analyzed in parallel with that from virus-infected cells. (a) Autoradiograms of virus-infected and uninfected cell DNA that was harvested at various time intervals, spotted onto nitrocellulose filter paper at various concentrations, and hybridized with ^{32}P -labeled AcMNPV DNA. Virus-infected cell DNA is shown in lanes: 1, 5.11×10^4 cells; 2, 3.83×10^4 cells; 3, 2.55×10^4 cells; and 4, 1.28×10^4 cells. Uninfected cell DNA is shown in lanes: 5, 2.55×10^4 cells; and 6, 1.28×10^4 cells. Time intervals (in hours [Hr]) for both virus-infected and uninfected cells are indicated to the left of the corresponding DNA spots (see the text for autoradiography). (b) After autoradiography, the DNA spots were excised and quantitated by liquid scintillation counting. Lanes: 1, liquid scintillation countings of virus-infected cell DNA from panel a, lane 1; 2, countings of panel a, lane 4; and 3, countings of uninfected cell DNA from panel a, lane 5. Time intervals (in hours [Hr]) for both virus-infected and uninfected cells are indicated to the left of the counts per minute (10^2).

that AcMNPV sequentially synthesized specific proteins in a cascade fashion.

When IPLB-LD-652Y cells were infected with AcMNPV, pulse-labeled with [^{35}S]methionine at various intervals p.i., and analyzed by SDS-PAGE, four viral-induced proteins were observed from 1 to 20 h p.i. by autoradiography (Fig. 5). Three early α proteins with approximate molecular weights of 46,500, 34,000, and 31,000 were detected from 1 to 4 h p.i. One unique protein with a molecular weight of 53,000 was also observed. This protein was never detected in replicate protein gels of AcMNPV-infected TN-368 cells. These four proteins were synthesized in increasing amounts from 4 to 12 h p.i. From 16 to 20 h p.i., a significantly lower percentage of radiolabel incorporation was observed in protein fractions from AcMNPV-infected IPLB-LD-652Y cells (data not shown). By 24 to 28 h p.i., both host and viral protein synthesis was completely suppressed. Three of the four proteins observed from 1 to 20 h p.i. had molecular weights like those reported by Wood (31), who used a clone of AcMNPV (1A) in TN-368 cells and an *S. frugiperda* cell line, IPLB-SF-21AE, and like those reported here for TN-368 cells infected with a plaque isolate of AcMNPV (6R) (compare Fig. 5 to Fig. 4). These results suggested that AcMNPV absorbed, penetrated, and initiated synthesis of some proteins in the IPLB-LD-652Y cell line.

DISCUSSION

Several gypsy moth cell lines have been described that vary in susceptibility to viral infection. Some supported complete replication of a variety of insect viruses but not that of LdMNPV (24), while others supported only those NPVs having a broad host range (11; R. H. Goodwin, unpublished observations). In each instance, the assessment of permissiveness or nonpermissiveness for a particular cell-virus system was based on microscopic examination

after infection. Hence, the criterion for permissiveness in gypsy moth cells was the ability to yield PIBs.

Recently, Carpenter and Bilimoria (4) described a model with SfMNPV, which replicated permissively in the homologous fall armyworm cell line IPLB-SF-21AE but semipermissively in the cabbage looper cell line TN-368. Their data showed that SfMNPV replication in TN-368 cells was aborted prior to the virion assembly stage. The criteria for viral replication in the semipermissive cell line were based on: (i) infection kinetic studies, which revealed a 700-fold lower yield of infectious progeny virus, (ii) cytopathology, in which SfMNPV-infected TN-368 cells produced classical cytopathic effects characteristic of NPV infections, and (iii) electron microscopic examination of infected TN-368 cells, which revealed nuclei containing virogenic stroma, aberrant and incomplete virions, and the absence of enveloped virus particles or PIBs.

The molecular mechanisms regulating permissiveness or nonpermissiveness of a given cell line for a particular virus have not been demonstrated for any baculovirus-cell system. In this report, we have shown that a gypsy moth cell subline, previously described as a nonpermissive cell line for AcMNPV, was, in fact, semipermissive. Our results, with a nick-translated AcMNPV DNA probe, showed that AcMNPV DNA synthesis occurred in the gypsy moth cell line, and the rate of synthesis approximated that observed in the permissive TN-368 cell line (32; J. T. McClintock, unpublished observations) and the IPLB-SF-21AE cell line (30).

Although AcMNPV DNA synthesis occurred in the gypsy moth cell line, protein synthesis was aborted during the α phase. During the α phase of protein synthesis in AcMNPV-infected IPLB-LD-652Y cells, two major differences from the permissive cell line (TN-368) were detected. An AcMNPV early 46,500-molecular-weight protein was synthesized at higher levels in the semipermissive line than in

FIG. 4. Autoradiographic comparison of electrophoretically separated [^{35}S]methionine-labeled polypeptides from AcMNPV-infected TN-368 cells labeled at various times p.i. Infected cells were radiolabeled for 2 h at 2-h intervals from 1 to 20 h p.i. Two additional samples were collected from 34 to 36 h and 46 to 48 h p.i. The numbers above the wells indicate the time (in hours after infection) at which the pulses were initiated (first number) and terminated (second number). The well marked Cell shows the protein profile of uninfected TN-368 cells labeled from 22 to 24 h p.i. The arrows on the right of the autoradiogram indicate the apparent molecular weight (10^3) of viral-induced polypeptides. OB, Occlusion body protein (polyhedrin). The dots (●) on the left indicate the four α proteins with apparent molecular weights of 46,500, 35,000, 34,000, and 31,000. The numbers on the left refer to the standard molecular weight (MW) markers (10^3).

the permissive line, suggesting a more stable transcript or more efficient transcription and translation of this particular protein or both. Alternatively, the 46,500-molecular-weight protein was modified or degraded in the permissive line but not in the semipermissive cell line.

Secondly, a 53,000-molecular-weight protein was detected in this semipermissive system which was not detected in either AcMNPV-infected TN-368 cells or LdMNPV-infected IPLB-LD-652Y cells. This protein may be related to one found by Wood et al. (32) in AcMNPV-infected TN-368 cells grown at high cell densities. It may belong to a class of proteins synthesized under suboptimal conditions (2).

Current evidence suggests that protein synthesis in NPV-infected cells occurs in a coordinately regulated cascade and that temporal and protein abundance controls are operating in the infected cells (7, 19). We have divided the replicative cycle into early, intermediate, and late phases based on the synthesis of certain infected-cell proteins in each phase and emphasize that each group of proteins is synthesized prior to the induction of proteins in subsequent phases (19). That only three of four early α proteins were synthesized in AcMNPV-infected gypsy moth cells may mean that a full

complement of proteins for each phase is required for the induction of subsequent phases. Alternatively, it may be that only a portion of the AcMNPV genome was activated. The transition from each phase may also be dependent on the synthesis of progeny viral DNA, since the initiation of viral DNA synthesis begins after the induction of the early α -phase polypeptides.

DNA methylation is generally associated with genome inactivity, whereas low levels of DNA methylation occur at those domains of the genome being transcribed (8). DNA methylation has also been shown to be important in determining permissive and nonpermissive infections (6) and virus latency (33). We investigated the possibility that host-induced methylation of viral DNA operated as a mechanism regulating expression of the fourth early α transcript. However, we have not found the viral DNA to be significantly methylated at the viral restriction sites recognized by the isoschizomers *HpaII* and *MspI*.

Further work on the factors regulating the permissive, semipermissive, or nonpermissive replicative cycle of AcMNPV in homologous or heterologous cell lines may improve our understanding of the factors involved in regu-

FIG. 5. SDS-polyacrylamide slab gel autoradiogram of AcMNPV-infected gypsy moth cells, IPLB-LD-652Y, pulse-labeled with [35 S]methionine at various intervals p.i. Uninfected control (C) and virus-infected (V) cells were compared at each time interval tested. The viral-induced proteins are indicated on the right with their corresponding apparent molecular weights (10^3). The numbers above the wells indicate the time (in hours after infection) at which the pulses were initiated (first number) and terminated (second number). The numbers along the left refer to the standard molecular weight markers (10^3). Note the absence of proteins, either of cell or viral origin, in IPLB-LD-652Y cells infected with AcMNPV and pulse-labeled from 24 to 28 and 48 to 52 h p.i.

lating control mechanisms during NPV replication. The gene(s) governing host range and virulence could then be manipulated in an intelligent and predictable fashion for a given insect host.

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