Viral and Host Cellular Transcription in *Autographa californica* Nuclear Polyhedrosis Virus-Infected Gypsy Moth Cell Lines

DAVID GUZO,* HAROLD RATHBURN, KIM GUTHRIE, AND EDWARD DOUGHERTY

Insect Biocontrol Laboratory, USDA-Agricultural Research Service, Beltsville, Maryland 20705-2350

Received 15 November 1991/Accepted 7 February 1992

Infection of two gypsy moth cell lines (IPLB-Ld652Y and IPLB-LdFB) by the *Autographa californica* multiple-enveloped nuclear polyhedrosis virus (AcMNPV) is characterized by extremely attenuated viral protein synthesis followed by a total arrest of all viral and cellular protein production. In this study, AcMNPV- and host cell-specific transcription were examined. Overall levels of viral RNAs in infected gypsy moth cells were, at most measured times, comparable to RNA levels from an infected cell line (TN-368) permissive for AcMNPV replication. Northern blot (RNA) analyses using viral and host gene-specific probes revealed predominantly normal-length virus- and cell-specific transcripts postinfection. Transport of viral RNAs from the nucleus to the cytoplasm and transcript stability in infected gypsy moth cells also appeared normal compared with similar parameters for AcMNPV-infected TN-368 cells. Host cellular and viral mRNAs extracted from gypsy moth and TN-368 cells at various times postinfection and translated in vitro yielded similar spectra of host and viral proteins. Treatment of infected gypsy moth cells with the DNA synthesis inhibitor aphidicolin eliminated the total protein synthesis shutoff in infected IPLB-LdFB cells but had no effect on protein synthesis inhibition in infected IPLB-Ld652Y cells. The apparent selective block in the translation of viral transcripts early in infection and the absence of normal translation or transcription of host cellular genes at later times is discussed.

The nuclear polyhedrosis viruses (subgroup A, family *Baculoviridae*) are large double-stranded DNA genome viruses which primarily infect insects. The *Autographa californica* multiple-enveloped nuclear polyhedrosis virus (AcMNPV) is the prototype for this virus group, and considerable information on its replicative cycle has been accumulated (for reviews, see references 10 and 33). Normally, replication of AcMNPV in permissive cell lines is regulated by the sequential activation of early, late, and very late viral genes (11). The products of some early genes are also believed to be essential for subsequent viral gene expression (2, 15). Concomitant with increasing expression of the AcMNPV genome, levels of some host cellular mRNAs are significantly reduced (24, 31) and host cellular protein synthesis is gradually inhibited.

The pathogenicity of many NPVs for a large number of economically important lepidopteran species has prompted interest in these viruses as potential biological pesticides (3, 8). The commercial production of these agents has, however, been hindered by the narrow host range of many NPVs. Previous studies on baculovirus host specificity were conducted primarily to satisfy safety concerns and have concentrated on identifying the barriers to AcMNPV replication in nonpermissive insect and vertebrate cell lines. Rice and Miller (27), for example, determined that some early viral genes are transcribed in AcMNPV-infected *Drosophila melanogaster* DL-1 cells but late viral promoter activity is not observed. However, this study was not conclusive and represented a minimal characterization of the viral RNA in infected cells. Additional studies (1, 26), using recombinant baculoviruses containing the *Escherichia coli* β -galactosidase gene under control of the AcMNPV polyhedrin promoter, indicate that expression of late baculoviral genes (e.g., the polyhedrin gene) in nonpermissive dipteran cells is

restricted and promoter dependent. Factors which restrict the host ranges of baculoviruses within the lepidoptera are, however, not understood.

Studies in our laboratory have concentrated on identifying the barriers to replication of AcMNPV in an insect, *Lymantria dispar* (the gypsy moth), not normally infected by this virus. Two cell lines derived from the gypsy moth, the ovarian line IPLB-Ld652Y (subsequently abbreviated as Ld652Y) and the larval fat body cell line IPLB-LdFB (subsequently abbreviated as LdFB) support a limited degree of viral protein synthesis as measured by pulse-labelling with [³⁵S]methionine followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. A total shutoff of all cellular and viral protein synthesis occurs at 20 to 24 h after AcMNPV infection in Ld652Y cells and after 48 h postinfection (p.i.) in LdFB cells. We demonstrate here that normal or elevated levels of viral transcripts and predominantly normal levels of cellular transcripts were produced in infected cells. However, translation of some virus-specific mRNAs initially, and of all transcripts at late times p.i., was inhibited.

MATERIALS AND METHODS

Cell lines and virus. Three cell lines were used in this study. The *L. dispar* cell line Ld652Y (14), the *L. dispar* fat body cell line LdFB (21), and the *Trichoplusia ni* line TN-368 (17) were maintained as previously described (13, 21). The AcMNPV clone 6R (23) was used in all infection studies.

Cells were generally infected with AcMNPV extracellular virus at a multiplicity of infection of 10 tissue culture infective doses at 50% infection per cell. After a 1-h infection period, the virus inoculum was removed and the cells were rinsed twice with sterile Lockes saline and refed fresh tissue culture medium. Mock-infected cells received identical treatments except that no virus was present.

RNA isolations. Total cellular RNAs were isolated from

* Corresponding author.

uninfected and AcMNPV-infected cells by a previously described method (9). Briefly, cells (in 175-cm² flasks) were rinsed with sterile Lockes saline and solubilized directly by the addition of 3 ml of a disruption buffer composed of 8 M guanidine hydrochloride, 0.3 M Na acetate, and 1% sarcosine. The solution was centrifuged at 12,000 × *g* for 10 min, the supernatant was removed, and RNAs were precipitated with one-half volume of 100% ethanol at -20°C for 30 min. Samples were centrifuged at 12,000 × *g* for 20 min, and the RNA pellets were resuspended in 0.5 ml of 8 M guanidine hydrochloride-0.3 M Na acetate. Samples were reprecipitated with one-half volume of 100% ethanol and stored at -20°C until used.

Cytoplasmic RNAs were isolated as described by Smith et al. (30). The remaining pelleted nuclei were solubilized in disruption buffer, and nuclear RNAs were extracted as described for total cellular RNAs. Total mRNAs from infected and uninfected cells were isolated by using the Fast Track mRNA isolation kit (Invitrogen, San Diego, Calif.) according to the manufacturer's instructions. Total cellular mRNAs were translated by using a rabbit reticulocyte lysate system (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and [³⁵S]methionine according to the manufacturer's protocol.

RNAs were separated for Northern blot (RNA) analysis by electrophoresis in 2.2 M formaldehyde denaturing gels run in 0.2 M morpholinepropanesulfonic acid (MOPS) buffer (22). RNA molecular weight (MW) standards (RNA ladder; Bethesda Research Laboratories) were coelectrophoresed to determine viral and host mRNA MWs. After electrophoresis, gels were blotted onto Nytran (Schleicher and Schuell, Keene, N.H.) filters, air dried for 1 h, and baked at 80°C for 2 h. RNA samples were also dot spotted onto nitrocellulose filters, air dried for 1 h, and baked at 80°C for 2 h.

DNA probes. Total AcMNPV DNA was isolated from sucrose gradient-purified viral occlusion bodies as previously described (23). The viral gene-specific probes IE-1 (plasmid pIE-1) (15) and IE-N (plasmid pPstI-N) (2) were generously provided by Linda Guarino, Texas A and M University, College Station, Tex. The AcMNPV major capsid protein gene (plasmid pSTSNM) (32) was kindly provided by Lois Miller, University of Georgia, Athens, Ga. A 385-bp fragment of the AcMNPV polyhedrin gene spanning the region from the *Hind*III site to the *Kpn*I site (18) was cloned into Bluescript KS+ (Stratagene, La Jolla, Calif.) and propagated in *E. coli* JM109 by the method of Chung and Miller (6). The α -tubulin and ribosomal p49 genes (cloned into pGem 4 blue vectors, designated p4-aT and pDrm114, respectively, and propagated in JM109) were generously provided by Mary Lou Pardue, Massachusetts Institute of Technology, Cambridge, Mass. DNA probes were labelled by nick translation (28) with [α -³²P]dCTP to specific activities of >10⁸ cpm/ μ g.

Inhibitors. The viral and cellular DNA synthesis inhibitor aphidicolin has previously been used to distinguish between early viral gene activity (not requiring viral DNA synthesis) and late baculoviral gene functions (25, 27). In this study, aphidicolin (Sigma Co.) was used at 40 μ g/ml of tissue culture medium and was added to cultures 1 h after AcMNPV infection. The transcription inhibitor actinomycin D (Sigma Co.) was used at 10 μ g/ml and added to cell cultures at various times p.i. The stability of viral transcripts was analyzed by probing dot blots of total cellular RNAs with radiolabelled whole viral DNA at 1, 2, 4, and 6 h after the addition of inhibitor.

Protein gels and Western blots (immunoblots). Protocols

FIG. 1. Comparisons of AcMNPV-specific RNA levels in infected TN-368, LdFB, and Ld652Y cells. Dot blots of total RNAs (20 μ g per spot) extracted from mock-infected (M) and AcMNPV-infected cells at various times (hours p.i. are indicated at the left of each row) were hybridized to radiolabelled AcMNPV genomic DNA. The numbers of counts per minute bound to each spot are listed at the right of the blots.

for the radiolabelling of tissue culture cells with [³⁵S]methionine (40 μ Ci/ml), as well as protocols for the preparation of SDS-polyacrylamide gels and cellular protein samples, were as previously described (23). After electrophoresis, gels were electroblotted onto nitrocellulose and probed with the anti-polyhedrin monoclonal antibody designated 1003 (kindly provided by Clinton Kawanishi, EPA, Research Triangle Park, N.C.). Characteristics of this particular monoclonal antibody have been previously described (19).

RESULTS

Levels of AcMNPV-specific RNAs in infected gypsy moth and TN-368 cells. The levels of AcMNPV-specific transcripts in infected gypsy moth and TN-368 cells were initially quantitated by simultaneously probing dot blots of total cellular RNAs (25 μ g per spot) with ³²P-labelled AcMNPV DNA (Fig. 1). The spots were subsequently excised, and the hybridized radioactivity was counted. Significant levels of AcMNPV-specific RNAs were detected in infected Ld652Y, LdFB, and TN-368 cells, particularly at later times (24 to 72 h) p.i. Additionally, Northern blots of total RNAs extracted from AcMNPV-infected Ld652Y, LdFB, and TN-368 cells probed with labelled AcMNPV DNA yielded similar-MW spectra of viral RNAs in all three cell lines (data not shown).

MWs of AcMNPV-specific transcripts in infected gypsy moth cells are similar to those for infected TN-368 cells. To determine whether the MWs of viral gene-specific transcripts in infected gypsy moth cells were similar to those for infected TN-368 cells, Northern blots of total cellular RNAs were probed with the following ³²P-labelled viral gene-specific probes: IE-1 (Fig. 2A), IE-N (Fig. 2B), the AcMNPV major capsid protein gene (Fig. 2C), and the polyhedrin gene (Fig. 2D). The IE-1 and IE-N genes are early genes which are believed to code for important regulatory proteins essential for subsequent normal viral gene expression (2, 15,

FIG. 2. Northern blots of total RNAs extracted from mock-infected (M) and AcMNPV-infected (2, 6, 12, 24, 48, and 72 h p.i.) TN-368, LdFB, and Ld652Y cells. RNAs (25 μ g per lane) were fractionated on formaldehyde (2.2 M)-1.2% agarose gels and hybridized with the following radiolabelled AcMNPV gene-specific probes: IE-1 (A), IE-N (B), major capsid protein gene (C), and polyhedrin gene (D). RNA size standards (0.24, 1.35, 2.37, 4.40, and 7.46 kb) are indicated to the left of the blots, while arrowheads to the right indicate prominent viral transcripts of 1.5, 1.9, 2.1, and 4.1 kb (A); 650 bp and 1.3, 2.1, and 3.5 kb (B); 2.2 kb (C); and 1.2 kb (D).

16), the major capsid protein gene is an important late gene, and the polyhedrin gene codes for one of the most abundant very late viral proteins. Major transcripts of all three genes in infected Ld652Y, LdFB, and TN-368 cells were similar in MW at most measurement times (0 to 72 h p.i.) to previously reported values for these transcripts (2, 5, 30, 32). Several exceptions were, however, observed; these involve replacement of a 1.3-kb IE-N transcript normally present in infected TN-368 and LdFB cells at 24 h p.i. with a 1.2-kb transcript in infected Ld652Y cells (Fig. 2B) and the observation of three previously unreported transcripts of 650 bp, 2.1 kb, and 3.5 kb in Northern blots of RNAs from all three cell lines hybridized with the IE-N probe. The temporal pattern of expression of viral genes in infected gypsy moth cells was also similar, in most instances, to that observed with the permissive cell line. Transcription of viral genes in the IE-N gene region at 24 h p.i. in infected Ld652Y cells was, however, considerably reduced compared with transcript levels in infected TN-368 and LdFB cells. Also, transcripts of the major capsid protein were initially observed at 6 h p.i. in infected Ld652Y cells and not until 12 h p.i. in infected TN-368 and LdFB cells (Fig. 2C).

Given the similar MWs and temporal expression patterns of the polyhedrin gene in infected gypsy moth and TN-368 cells, it was of interest to determine more definitively whether polyhedrin transcripts were translated in infected gypsy moth cells. To measure translation, proteins from AcMNPV-infected TN-368, Ld652Y, and LdFB cells at 6, 12, 24, 48, and 72 h p.i. were electroblotted onto nitrocellulose and probed with anti-AcMNPV polyhedrin monoclonal antibody. Polyhedrin protein was detected only at 24, 48, and 72 h p.i. in the infected TN-368 cells; no signals were

detected in infected Ld652Y or LdFB cells at any time p.i. (Fig. 3).

Transport and stability of viral transcripts in gypsy moth cells. Nuclear processing and/or transport of viral and host transcripts has been shown to play a role in viral expression in several virus-host cell systems (4, 7). It was therefore conceivable that the absence of normal viral protein synthesis in infected gypsy moth cells could result from a breakdown in the transport of viral transcripts from the nucleus to the cytoplasm. To determine whether this was the case, nuclear and cytoplasmic total RNA fractions were extracted from infected TN-368, Ld652Y, and LdFB cells at a time (24 h p.i.) when extensive virus-specific transcripts were known to be present (Fig. 1 and 2), dot blotted onto Nytran filters, and probed with whole virus- or gene-specific probes (Fig. 4). In all cases, considerable virus-specific transcripts were

FIG. 3. Western blot of cellular proteins from mock-infected (M) and AcMNPV-infected (6, 12, 24, 48, and 72 h p.i.) TN-368, LdFB, and Ld652Y cells. Protein samples were subjected to SDS-PAGE in 10% acrylamide gels, electroblotted onto nitrocellulose filters, and probed with an anti-AcMNPV polyhedrin monoclonal antibody.

FIG. 4. Dot blots of nuclear (N) and cytoplasmic (C) RNAs (20 µg per spot) extracted from AcMNPV-infected (24 and 48 h p.i.) TN-368 (A), LdFB (B), and Ld652Y (C) cells. Blots were hybridized with radiolabelled AcMNPV genomic DNA.

present in cytoplasmic RNA fractions in both infected gypsy moth cells and infected TN-368 cells.

The stability of virus-specific transcripts in infected gypsy moth and TN-368 cells was analyzed by incubating infected cells at 12 h p.i. with actinomycin D for periods of 1, 2, 4, and 6 h. Because actinomycin D is an inhibitor of RNA synthesis, the decline in RNA levels over time in the presence of this inhibitor should be a measure of RNA stability. Total RNAs were extracted from infected cells at the end of each incubation period, blotted, and probed with labelled whole AcMNPV DNA (Fig. 5). The stability of virus-specific transcripts in infected gypsy moth cells did not appear to be significantly different from that in infected TN-368 cells as judged by dot blot hybridization intensities.

Analysis of host cellular transcription. Synthesis of host cell-specific proteins has previously been demonstrated to initially remain essentially normal in infected Ld652Y and LdFB cells, as judged by pulse-labelling with [³⁵S]methionine followed by SDS-PAGE and autoradiography. However, total inhibition of protein synthesis occurs abruptly at 16 to 20 h p.i. in Ld652Y cells (23) (see Fig. 7A and B) and after 48 h p.i. in LdFB cells (8a) (see Fig. 7C and D). It was therefore

FIG. 5. Stability of AcMNPV-specific transcripts after incubation of AcMNPV-infected cells with actinomycin D. AcMNPV-infected TN-368, LdFB, and Ld652Y cells (at 24 h p.i.) were incubated with the transcription inhibitor actinomycin D (10 µg/ml) for 1, 2, 4, and 6 h. Total RNAs were subsequently extracted from the actinomycin D-treated cells, dot blotted (20 µg per spot), and hybridized with radiolabelled AcMNPV genomic DNA.

FIG. 6. Northern blots of total RNAs extracted from mock-infected (M) and AcMNPV-infected (6, 12, 24, 48, and 72 h p.i.) TN-368, LdFB, and Ld652Y cells. Sample size and electrophoresis conditions were as described in the legend to Fig. 2. The blots were hybridized with the radiolabelled *D. melanogaster* α-tubulin gene. RNA size standards (in kilobases) are to the left of the blots.

of interest to determine whether transcription of host-specific genes was altered at various times before and after total protein synthesis inhibition. Northern blots of total RNAs extracted from uninfected and AcMNPV-infected TN-368 and gypsy moth cells at 2, 6, 12, 24, 48, and 72 h p.i. were subsequently probed with the cellular α-tubulin and ribosomal p49 genes. No consistent changes (decreases or increases) in levels of transcripts from these genes were observed for infected LdFB cells over the 72-h measurement period; however, cellular transcript levels in infected Ld652Y cells were greatly attenuated after 24 h p.i. Data for the α-tubulin gene-specific probe are illustrated in Fig. 6. Interestingly, in contrast to the marked decline in host mRNA levels observed previously at 12 to 18 h p.i. with AcMNPV-infected IPLB-Sf21 cells (references 25 and 31 and unpublished observations from our laboratory), levels of TN-368 cellular mRNAs remained essentially unchanged over the duration of the experiment.

Effects of aphidicolin on protein synthesis inhibition. To determine whether the AcMNPV infection-induced changes in gypsy moth cellular transcription and translation (as evidenced by the total cellular protein synthesis inhibition) were a function of early or late viral gene functions, infected cells were incubated with the DNA polymerase inhibitor aphidicolin. After AcMNPV infection (multiplicity of infection, 10) of Ld652Y and LdFB cells, some cultures were incubated in the presence of 40 µg of aphidicolin per ml. Cell proteins from infected cultures with and without aphidicolin were subsequently pulse-labelled with [³⁵S]methionine for 4-h periods at various times p.i. and subjected to SDS-PAGE and autoradiography (Fig. 7). Aphidicolin eliminated the total protein synthesis inhibition effect (as previously observed [8a]) as well as the synthesis of a 37,000-MW virus infection-specific protein in AcMNPV-infected LdFB cells (Fig. 7C and D) but had little or no effect on suppressing the synthesis of three virus infection-specific proteins (MWs of 34,000, 39,000, and 45,000) or on the subsequent protein synthesis inhibition in infected Ld652Y cells (Fig. 7A and B). It should be noted that aphidicolin treatment of uninfected cells did, by 96 h postincubation, also reduce total cellular protein synthesis; however, it did not totally eliminate it.

In vitro translation of viral and host cellular transcripts. The presence of apparently normal-length viral and cellular transcripts, combined with the absence of normal protein synthesis in infected gypsy moth cells, engendered the question of

FIG. 7. Autoradiogram of electrophoretically separated [35 S]-methionine-labelled proteins from AcMNPV-infected Ld652Y cells (A), aphidicolin-treated AcMNPV-infected Ld652Y cells (B), AcMNPV-infected LdFB cells (C), and aphidicolin-treated AcMNPV-infected LdFB cells (D). Untreated infected cells and infected cells treated with aphidicolin were labelled for alternate 4-h periods from 1 to 28 h p.i. and subsequently for 4-h periods at 44 to 48, 68 to 72, and 92 to 96 h p.i. Lanes marked Cell(1) and Cell(2) for untreated AcMNPV-infected cells show protein profiles for mock-infected cells at the beginning and end of each experiment, respectively, while lanes marked Cell(1) and Cell(2) for aphidicolin-treated infected cells indicate cell proteins from mock-infected cells incubated with aphidicolin for the duration of the experiment (96 h). Protein MW markers (10^3) are to the left of each autoradiogram. Arrowheads in panels A and B indicate virus-specific 34,000-, 39,000-, and 45,000-MW proteins, while the arrowhead in panel C indicates a viral infection-induced 37,000-MW protein.

whether these transcripts could be translated in vitro. To determine this, total mRNAs were isolated from AcMNPV-infected TN-368 and gypsy moth cells at times before and after total protein synthesis inhibition and were translated into a rabbit reticulocyte lysate in vitro translation system. Typical results are shown in Fig. 8. Similar patterns of virus-specific translation products were observed with both infected TN-368 and gypsy moth cells prior to and after the protein synthesis shutoff. Prominent viral infection-specific proteins with apparent MWs of 27,000, 33,000, 37,500, 40,000, 42,000, and 61,000 were observed with the three cell lines. Infected Ld652Y cells did, however, exhibit a number of viral infection-specific protein bands (those of MW 35,000 and 36,000) that were either present in smaller quantities or not detectable in the other cell lines. Some viral bands (those of MW 25,000

and 34,000) were also absent in infected Ld652Y cells. Possibly, higher levels of certain viral infection-induced proteins in Ld652Y cells are a result of the translation of elevated quantities of some AcMNPV-specific transcripts in this cell line or of the activation of minor or cryptic viral promoters. Also, in contrast to the absence of α -tubulin gene transcripts after 24 h p.i. (Fig. 6), the continued synthesis of some Ld652Y cell-specific proteins at 72 h p.i. indicated either that transcription of some cellular genes (other than the specific genes examined here) persisted for an extended period after viral infection-induced protein synthesis shutoff or that some cellular transcripts in infected cells could have prolonged half-lives. SDS-polyacrylamide gels of proteins synthesized in vitro were also electroblotted and probed with anti-polyhedrin monoclonal antibody to ascertain the presence of polyhedrin protein in infected Ld652Y and LdFB cells. The presence of polyhedrin protein was confirmed at 24 and 72 h p.i. for infected TN-368 and LdFB cells and at 12, 24, and 72 h p.i. for Ld652Y cells (Fig. 9).

DISCUSSION

The characterization of insect cell lines which support limited replication of certain baculoviruses can offer insights into some of the molecular barriers which restrict the host range of these viruses. Unlike permissive baculovirus-host cell systems in which a defined cascade of viral structural and nonstructural genes is activated, the AcMNPV-Ld652Y and -LdFB systems are characterized by an extremely attenuated level of viral protein synthesis followed by an abrupt and total shutoff of all protein production. In this study, we demonstrate that the absence of normal AcMNPV protein synthesis was not due to a lack of virus-specific transcription but instead could result from an inability of host gypsy moth cells to translate abundant viral mRNAs. Additionally, synthesis of a normal spectrum of viral gene products after in vitro translation of mRNAs from infected gypsy moth cells as well as the stable nature of these transcripts indicated that virus-specific mRNAs were neither excessively destabilized nor defective with regard to protein synthesis.

The transcription of AcMNPV genes occurred in the presence of normal cellular protein synthesis activity and subsequently in the absence of all detectable protein production at later times p.i. This initial period of normal cellular protein synthesis encompassed (in infected LdFB cells, for example) the transcription of early, late, and very late viral genes without the synthesis of the expected viral proteins. The translatability of viral transcripts in vitro (Fig. 8) and the absence of a block in the transport of viral mRNAs from the nucleus to the cytoplasm in infected cells (Fig. 4) implied a breakdown in the translation of some viral mRNAs in the presence of normal host cell protein synthesis. The mechanism(s) underlying this initial putative block in the translation of some viral transcripts is not known. Conceivably, successful interactions between some viral transcripts and host *L. dispar* translational factors may be lacking. Host cells might also be able to distinguish between host and some viral mRNAs by unique sequences that are present only in some viral transcripts. For example, late AcMNPV RNAs are initiated at an ATAAG-containing consensus sequence (24, 29). While the significance of this 5' sequence is still unclear, it is possible that infected gypsy moth cells are unable to properly recognize and translate mRNAs possessing these or similar virus-specific sequences.

It should be noted, however, that the block in the translation of viral mRNAs in infected gypsy moth cells (prior to

FIG. 8. Autoradiogram of electrophoretically separated [^{35}S]methionine-labelled in vitro translation products. Poly(A) $^{+}$ RNAs were extracted from AcMNPV-infected (6, 12, 24, and 72 h p.i.) TN-368 (A), LdFB (B), and Ld652Y (C) cells and incubated in a rabbit reticulocyte lysate in vitro translation mixture. Lanes marked Cell show protein synthesis in mock-infected cells. Arrowheads indicate MWs (10^3) of virus infection-specific proteins common to all three cell lines and specific for infected Ld652Y cells.

the total protein synthesis shutoff) is probably not a complete one. Pulse-labelling with [^{35}S]methionine followed by SDS-PAGE and autoradiography, for example, indicated that three virus infection-specific proteins were synthesized in infected Ld652Y cells and one protein species was synthesized in LdFB cells. The essentially normal kinetics of AcMNPV DNA replication in infected gypsy moth cells (8a, 23) indicates that the AcMNPV DNA polymerase is probably synthesized at normal levels, and transcription of late viral genes (e.g., the polyhedrin gene) also implies the presence of the virus-induced α -amanitin-resistant RNA polymerase (12, 20) as well as the presence of regulatory viral gene products (i.e., IE-1 and IE-N) responsible for controlling the normal cascade of viral gene activation. The greatly reduced level of viral protein production combined with the normal regulation of viral mRNA synthesis in these systems should make them excellent candidates for analysis of the viral elements and gene products which regulate the ordered transcription of baculoviral genes.

The total shutoff of all detectable viral (and cellular) protein synthesis observed after 16 h p.i. with Ld652Y cells and after 48 h p.i. with LdFB cells was also characterized by continued transcription of viral genes, translatability of virus transcripts in vitro, and transport of viral mRNAs from

nucleus to cytoplasm. The absence of any detectable cellular protein synthesis at these times was, however, indicative of an AcMNPV infection-induced block in a more fundamental aspect of protein synthesis, possibly at the translational level, in infected *L. dispar* cells.

In contrast to the essentially normal cascade of virus-specific transcription at all measurement times p.i. in both gypsy moth cell lines, differences between infected LdFB and Ld652Y cells were noted with regard to regulation of host mRNA synthesis. While host-specific mRNA levels in infected LdFB cells remained essentially constant at all measurement times p.i., substantial reductions in Ld652Y cell-specific transcripts were temporally correlated with the host protein synthesis shutoff. This data, combined with the inability of aphidicolin to eliminate the protein synthesis shutoff in infected Ld652Y cells and the continued synthesis of virus-specific transcripts, implied that an early viral gene product(s) may mediate protein synthesis shutoff by selectively degrading or destabilizing host transcripts or by inhibiting some essential transcriptional component(s). In contrast, the presence of essentially unchanged levels of host mRNAs after protein synthesis shutoff in infected LdFB cells and the translatability of host transcripts in vitro implied the presence of a translational block in cellular protein production. Unfortunately, while aphidicolin abrogated the total protein synthesis shutoff in LdFB cells, it also significantly reduced protein synthesis in mock-infected cells, and any conclusions concerning early versus late viral functions with regard to potential mechanisms of protein synthesis shutoff in this cell line cannot be made at present. Whether the differences in host cellular mRNA synthesis in the two cell lines indicate different susceptibilities to AcMNPV infection or are the product of differential viral gene expression is not known.

Interestingly, TN-368 cell-specific transcription remained essentially unaffected during the course of virus infection (Fig. 6). This is in marked contrast to the AcMNPV infection-induced inhibition of host mRNA levels in permissive IPLB-Sf21 cells which has been observed by us (unpublished

FIG. 9. Western blots of proteins synthesized by in vitro translation of total cellular mRNAs extracted from mock-infected (M) and AcMNPV-infected (6, 12, 24, and 72 h p.i.) TN-368, LdFB, and Ld652Y cells. Protein samples were subjected to SDS-PAGE in 10% acrylamide gels, electroblotted onto nitrocellulose filters, and probed with an anti-AcMNPV polyhedrin monoclonal antibody. MWs (10^3) are indicated at left. The arrow indicates the polyhedrin protein.

observations) and other researchers (25, 31). While results presented here show potential differences in the regulation of mRNA levels in cell lines permissive for AcMNPV replication, additional studies on a variety of NPV-infected cell lines need to be conducted before any definitive conclusions can be drawn.

The results of this and previous studies on AcMNPV replication in gypsy moth cells indicate that the barriers which restrict AcMNPV replication in the gypsy moth occur subsequent to a considerable amount of virus-specific gene activity. Unlike previous studies which suggested a promoter-dependent restriction of AcMNPV gene expression in nonlepidopteran host cells, *L. dispar* cellular DNA-dependent RNA polymerases and other transcriptional factors appear to efficiently recognize a broad spectrum of viral promoters and transcribe early to very late viral genes at levels equivalent to those in permissive cells. The presence of an intracellular environment in gypsy moth cells that is conducive to substantial AcMNPV biosynthetic activity is perhaps not too surprising, given the broad host range of AcMNPV in the lepidoptera. However, further studies on AcMNPV infection of cell lines from additional lepidopteran species which are not normally considered hosts for this virus need to be conducted to determine whether the results presented here are unique to gypsy moth cell lines or instead represent a more common phenomenon underlying AcMNPV host range barriers in the lepidoptera.

REFERENCES

1. Carbonell, L. F., M. J. Klowden, and L. K. Miller. 1985. Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. *J. Virol.* **56**:153-160.
2. Carson, D. D., L. A. Guarino, and M. D. Summers. 1988. Functional mapping of an AcMNPV immediately early gene which augments expression of the IE-1 transactivated 39K gene. *Virology* **162**:444-451.
3. Carter, J. B. 1984. Viruses as pest-control agents. *Biotechnol. Genet. Eng. Rev.* **1**:375-419.
4. Castiglia, C. L., and S. J. Flint. 1983. Effects of adenovirus infection on rRNA synthesis and maturation in HeLa cells. *Mol. Cell. Biol.* **3**:662-670.
5. Chisholm, G. E., and D. J. Henner. 1988. Multiple early transcripts and splicing of the *Autographa californica* nuclear polyhedrosis virus IE-1 gene. *J. Virol.* **62**:3193-3200.
6. Chung, C. T., and R. H. Miller. 1988. A rapid and convenient method for the preparation and storage of competent bacterial cells. *Nucleic Acids Res.* **16**:3580.
7. DeMarchi, J. M. 1983. Nature of the block in expression of some early genes in cells abortively infected with human cytomegalovirus. *Virology* **129**:287-297.
8. Dougherty, E. M. 1987. Insect viral control agents. *Dev. Ind. Microbiol.* **28**:63-75.
- 8a. Dougherty, E. M., et al. Submitted for publication.
9. Evans, R., and S. J. Kamdar. 1990. Stability of RNA isolated from macrophages depends on the removal of an RNA-degrading activity early in the extraction procedure. *BioTechniques* **8**:357-362.
10. Faulkner, P., and E. P. Carstens. 1987. An overview of the structure and replication of baculoviruses, p. 1-20. In W. Doerfler and P. Bohm (ed.), *The molecular biology of baculoviruses*. Springer-Verlag, Berlin.
11. Friesen, P. D., and L. K. Miller. 1986. The regulation of baculovirus gene expression. *Curr. Top. Microbiol. Immunol.* **131**:31-49.
12. Fuchs, L. Y., M. S. Woods, and R. F. Weaver. 1983. Viral transcription during *Autographa californica* nuclear polyhedrosis virus infection: a novel RNA polymerase induced in infected *Spodoptera frugiperda* cells. *J. Virol.* **41**:641-646.
13. Goodwin, R. H., and J. R. Adams. 1980. Nutrient factors influencing viral replication in serum-free insect cell line culture, p. 493-509. In E. Kurstak, K. Maramorosch, and A. Dubendorfer (ed.), *Invertebrate systems in vitro*. Elsevier/North-Holland Biomedical Press, Amsterdam.
14. Goodwin, R. H., G. J. Tompkins, and P. McCawley. 1977. Gypsy moth cell lines divergent in viral susceptibility. I. Culture and identification. *In Vitro (Rockville)* **14**:485-493.
15. Guarino, L. A., and M. D. Summers. 1986. Functional mapping of a *trans*-activating gene required for expression of a baculovirus delayed-early gene. *J. Virol.* **57**:563-571.
16. Guarino, L. A., and M. D. Summers. 1987. Nucleotide sequence and temporal expression of a baculovirus regulatory gene. *J. Virol.* **61**:2091-2099.
17. Hink, W. F. 1970. Established insect cell line from the cabbage looper, *Trichoplusia ni*. *Nature (London)* **226**:466-467.
18. Hooft van Iddekinge, B. J. L., G. E. Smith, and M. D. Summers. 1983. Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. *Virology* **131**:561-565.
19. Huang, Y.-S., P. C. Hu, and C. Y. Kawanishi. 1985. Monoclonal antibodies identify conserved epitopes on the polyhedrin of *Heliothis zea* nuclear polyhedrosis virus. *Virology* **143**:380-391.
20. Huh, N. E., and R. F. Weaver. 1990. Identifying the RNA polymerases that synthesize specific transcripts of the *Autographa californica* nuclear polyhedrosis virus. *J. Gen. Virol.* **71**:195-201.
21. Lynn, D. E., E. M. Dougherty, J. T. McClintock, and M. Loeb. 1988. Development of cell lines from various tissues of lepidoptera, p. 239-242. In Y. Kuroda, E. Kurstak, and K. Maramorosch (ed.), *Invertebrate and fish tissue culture*. Japan Scientific Press, Tokyo.
22. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. McClintock, J. T., E. M. Dougherty, and R. M. Weiner. 1986. Semipermissive replication of a nuclear polyhedrosis virus of *Autographa californica* in a gypsy moth cell line. *J. Virol.* **57**:197-204.
24. Miller, L. K. 1988. Baculoviruses as gene expression vectors. *Annu. Rev. Microbiol.* **42**:177-199.
25. Ooi, B. G., and L. K. Miller. 1988. Regulation of host RNA levels during baculovirus infection. *Virology* **166**:515-523.
26. Pennock, G. D., C. Shoemaker, and L. K. Miller. 1984. Strong and regulated expression of *Escherichia coli* β -galactosidase in insect cells with a baculovirus vector. *Mol. Cell. Biol.* **4**:399-406.
27. Rice, W. C., and L. K. Miller. 1986. Baculovirus transcription in the presence of inhibitors and in nonpermissive *Drosophila* cells. *Virus Res.* **6**:155-172.
28. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**:237-251.
29. Rohrmann, G. F. 1986. Polyhedrin structure. *J. Gen. Virol.* **67**:1499-1513.
30. Smith, G. E., J. M. Vlak, and M. D. Summers. 1983. Physical analysis of *Autographa californica* nuclear polyhedrosis virus transcripts for polyhedrin and 10,000-molecular-weight protein. *J. Virol.* **45**:215-225.
31. Talhouk, S. N., and L. E. Volkman. 1991. *Autographa californica* M nuclear polyhedrosis virus and cytochalasin D: antagonists in the regulation of protein synthesis. *Virology* **182**:626-634.
32. Thiem, S. M., and L. K. Miller. 1991. Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **63**:2008-2018.
33. Volkman, L. E., and D. L. Knudson. 1986. *In vitro* replication of baculoviruses, p. 109-129. In R. R. Grandados and B. A. Federici (ed.), *The biology of baculovirus*. CRC Press Inc., Boca Raton, Fla.