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Effects of Baculovirus Infection on IE1-Mediated Foreign Gene Expression in Stably Transformed Insect Cells

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Previously, we produced transformed insect cell lines that can express a selected foreign protein constitutively, in the absence of baculovirus infection (D. L. Jarvis, J. G. W. Fleming, G. R. Kovacs, M. D. Summers, and L. A. Guarino, Bio/Technology 8:950–955, 1990). These cells contain stably integrated copies of chimeric genes consisting of the promoter from an immediate-early baculovirus gene, IE1, and the sequences encoding either human tissue plasminogen activator or *Escherichia coli* β-galactosidase. Transcription of the integrated genes in these cells is specifically controlled by the IE1 promoter. The purpose of this study was to determine how baculovirus infection influences IE1-mediated foreign protein production by these stably transformed insect cell lines. The results showed that viral infection transiently stimulated and then strongly inhibited the production of both tissue plasminogen activator, a secreted protein, and β-galactosidase, an intracellular protein. These effects reflected virus-induced changes in the steady-state levels of RNA produced by the integrated genes. Transient assays showed that expression of the viral IEN gene alone could account for the increased levels of RNA observed early in infection. The precise mechanism accounting for the decreased levels of RNA observed later in infection was not determined. However, we obtained evidence that the native IE1 promoter remains active throughout infection, which suggested indirectly that the integrated IE1 promoter is transcriptionally inactivated at late times of baculovirus infection. Thus, the same promoter behaved quite differently late in infection, depending on its local environment. Neither methylation nor degradation appeared to be responsible for inactivating IE1-mediated expression of the integrated genes. The significance of these results with respect to the baculovirus-host interaction and the practical applications of stably transformed insect cell lines are discussed.

The baculovirus *Autographa californica* multicapsid nuclear polyhedrosis virus (Ac*M*NPV) is a rod-shaped DNA-containing virus that infects lepidopteran insects in nature. In the laboratory, several different lepidopteran insect cell lines, including *Spodoptera frugiperda* IPLB-Sf21-AE (44), can be used as hosts for Ac*M*NPV. During the past decade, the Ac*M*NPV-Sf cell system has been successfully developed as a tool for the high-level expression of foreign gene products in a eucaryotic host (reviewed in references 35–37). This was accomplished by taking advantage of the fact that a highly expressed viral gene, polyhedrin, is dispensable for the growth of Ac*M*NPV in cultured insect cells (40). Thus, one can replace the polyhedrin coding sequence with the sequence encoding a desired foreign protein and isolate a viable recombinant virus. The recombinant can then be used to infect insect cells, and the foreign gene will be expressed at late times of infection under the transcriptional control of the viral polyhedrin promoter, which usually results in the production of large amounts of the foreign protein.

Widespread use of the baculovirus expression system has provided valuable information on the protein-processing capabilities of lepidopteran insect cells, particularly regarding their ability to process secretory pathway proteins derived from higher eucaryotes (reviewed in reference 26). Generally, these studies have shown that these proteins can be appropriately modified and transported by insect cells. It should be noted, however, that the results of most (2, 3, 20, 32), but not all (10–12) of these studies indicate that the precise nature of the N-glycosylation pathway in insect cells differs from the one found in higher eucaryotes (reviewed in reference 29).

Studies of foreign protein processing in the baculovirus system also have yielded valuable information on the baculovirus-host cell interaction. For example, these studies have provided evidence that baculovirus infection can influence the modification (10, 11) and transport (23, 25) of proteins synthesized by the host cell secretory pathway. The idea that baculovirus infection might impair the function of the secretory pathway was originally proposed in a study of the expression and processing of human tissue plasminogen activator (t-PA), in which it was observed that the efficiency of t-PA secretion decreased as the viral infection progressed (25). This idea was supported by the results of a subsequent study, which showed that human t-PA is secreted more efficiently and quickly by uninfected insect cells than by insect cells infected with a recombinant baculovirus (23). These findings are not peculiar to t-PA, because the efficiency of human interleukin 2 secretion by recombinant baculovirus-infected insect cells also decreases with increasing time of infection (20a). Moreover, it has been suggested that the relatively inefficient and slow processing of influenza virus hemagglutinin protein in baculovirus-infected insect cells (33) might reflect an inhibition of secretory pathway function during baculovirus infection.

The expression of t-PA in uninfected insect cells was accomplished by isolating stably transformed insect cell lines that express the human t-PA gene constitutively under the control of the promoter from the Ac*M*NPV immediate-early gene, IE1 (17, 18, 21, 23, 24). The chimeric gene containing the IE1 promoter and the t-PA coding sequence is stably integrated into the genomes of these cells, and transcription is specifically controlled by the IE1 promoter. The availability of this and another transformed insect cell line, which expresses *Escherichia coli* β-galactosidase (β-gal),

provided a unique opportunity to address some interesting questions about the baculovirus-host cell interaction. Some of these questions were answered by this study, which was designed to determine how baculovirus infection influences the IE1-mediated expression and processing of foreign gene products by transformed insect cells.

MATERIALS AND METHODS

Cells and viruses. The Sf9 subclone of the IPLB-SF21-AE cell line (44) was used to produce transformed insect cell lines that constitutively express *E. coli* β-gal or human t-PA under the control of the Ac*M*NPV IE1 promoter. The isolation and characterization of these cell lines, which were designated IE1FB2 and IE1TPA15, respectively, have been described (23, 24). It is important to note that we have used S1 nuclease analyses to show that transcription of the integrated genes initiates specifically at the consensus early start site (CAGT) in the IE1 promoter in these cells (23 and this study). Furthermore, Southern blots have shown that the sequences from positions −600 to +36 from the IE1 gene are intact in IE1FB2 cell DNA, which indicates that the promoter and upstream regulatory sequences remained intact after integration. Thus, there is substantial evidence to support the conclusion that the β-gal and t-PA genes in these transformed cells are transcribed under IE1 control. All cells used in this study were maintained as adherent cultures at 28°C in TNM-FH medium (43) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (Hazelton Research Products, Inc., Lenexa, Kans.), 1.25 μg of amphotericin B per ml (Sigma Chemical Co., St. Louis, Mo.), and 25 μg of gentamicin per ml (Sigma). The radiolabeling medium was methionine-free Grace's medium (43) supplemented with the same concentrations of heat-inactivated fetal bovine serum and antibiotics. The E2 strain of Ac*M*NPV (42) was propagated and titrated by plaque assay in Sf9 cells as described by Summers and Smith (43).

Infections and transfections. Large pools of IE1FB2 or IE1TPA15 cells were split at equal densities (1×10^6 to 7.5×10^6 cells) into multiple 25- or 75-cm^2 flasks (Corning, Inc., Corning, N.Y.) and incubated for 1 to 24 h at 28°C, the medium was drained, and the cells were mock infected with TN-MFH or infected with Ac*M*NPV. After infection, the cells were incubated for various times at 28°C, according to the requirements of the experiment, and then protein, RNA, and DNA analyses were done as described below.

For transfections, large pools of IE1FB2 or IE1TPA15 cells were split at a density of 10^6 cells per well into six-well plates (Corning) and allowed to attach for 1 h at 28°C. The medium was drained, and the cells were transfected in triplicate with 5 μg of CsCl-purified plasmid DNA, Ac*M*NPV DNA, or calf thymus DNA per well by using the modified calcium phosphate method described by Summers and Smith (43). The plasmids used for transfections contained the following Ac*M*NPV genes: IE0 (pGC123) (8), IE1 (pAcIE1) (17), IEN (pPstI-N) (4), or a functionally inactivated version of IEN [pPstI-N-Bgl(−)] (4). After transfection, the cells were washed and incubated at 28°C until protein analyses were done as described below.

Protein analyses. Protein analyses were performed as follows. At various times postinfection or at 20 h posttransfection, cells were pulse-labeled for 4 h with 100 μCi of Tran^{35}S-Label (ICN Radiochemicals, Irvine, Calif.) per ml of radiolabeling medium, and then the medium was harvested and clarified for 1 min in a microcentrifuge. The supernatant was harvested as the extracellular fraction, and Nonidet P-40 was added to a final concentration of 1% (vol/vol). The cell pellet was resuspended in extraction buffer (50 mM Tris, pH 8.0, 100 mM NaCl, 1% [vol/vol] Nonidet P-40, 500 Kallikrein inactivating units of aprotinin per ml [Boehringer Mannheim Corporation, Indianapolis, Ind.]) and added back to the cells remaining in the flask. After 20 min on ice, the extraction buffer was harvested and then clarified for 10 min in a microcentrifuge, and the supernatant was recovered as the intracellular fraction. Extracellular and intracellular fractions were immunoprecipitated with goat anti-t-PA (American Diagnostica, New York, N.Y.) or mouse anti-β-gal (Promega Corporation, Madison, Wis.) as described previously (25), and immunoprecipitated proteins were resolved by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (34). The gels were stained with Coomassie blue, destained, dried, and autoradiographed. Finally, t-PA and β-gal bands were excised from the gels, the gel slices were solubilized, and radioactivity was measured in a liquid scintillation counter as described previously (22).

Isolation and analysis of total cellular RNA. Total cellular RNA was prepared by the method of Chirgwin and coworkers (7). Briefly, mock-infected or infected cells were washed with ice-cold TBS (25 mM Tris, pH 7.4, 140 mM NaCl) and resuspended in a solution of 4 M guanidine isothiocyanate, 50 mM Tris (pH 7.5), 10 mM EDTA, 2% (wt/vol) Sarkosyl, and 140 mM β-mercaptoethanol. High-molecular-weight DNA was sheared by trituration through a 22-gauge needle, and then solid CsCl was added (0.4 g/ml of lysate), and the resulting solution was layered onto 5.7 M CsCl cushions. The gradients were centrifuged in a Beckman SW60 rotor at 30,000 rpm for 16 h, and the RNA pellets were dissolved in TE buffer (10 mM Tris, pH 8.0, 1.0 mM EDTA), extracted with phenol-chloroform and chloroform, and precipitated with ethanol. Ethanol precipitates were redissolved in TE and quantitated by spectrophotometry. For RNA stability measurements, infected cells were incubated in the presence of 10 μg of actinomycin D (Sigma) per ml of TN-MFH for various time periods, beginning at 4 h postinfection, prior to isolation of total cellular RNA by this same method.

S1 nuclease analysis of total cellular RNA was done essentially as described by Weaver and Weissmann (45) and Guarino and Summers (17). The t-PA probe was a 759-bp *Bam*HI-*Bgl*II fragment of pIE1TPA (23), which generates a 203-bp protected fragment with transcripts initiating at the early motif (CAGT at position −51) in the IE1 promoter. The β-gal probe was an 848-bp *Bam*HI-*Ban*I fragment of pIE1FB (23), which generates a 290-bp protected fragment, and the IE1/IE0 probe was an 1,195-bp *Hin*fI fragment of pGC123 (8), which generates 341- or 531-bp protected fragments with IE1 or IE0 RNA, respectively. pIE1TPA, pIE1FB, or pAcIE1 was digested with *Bgl*II, *Ban*I, or *Hin*fI, respectively, and dephosphorylated with calf alkaline phosphatase. The t-PA and β-gal probes were subjected to secondary digestion with *Bam*HI and *Eco*RI, respectively, and then all probes were gel purified twice. Probe fragments were recovered from the gel and 5′ end labeled with T4 polynucleotide kinase under standard reaction conditions (39). Labeled probes were precipitated twice with ethanol and quantitated, and 20,000 to 100,000 cpm was used for S1 nuclease reactions with 10 to 20 μg of total cellular RNA. Hybridization was done overnight at 50°C in 80% (vol/vol) formamide, and the products were digested with S1 nuclease and analyzed by PAGE in the presence of 7 M urea as described previously (17).

Isolation and analysis of genomic DNA. Genomic DNA was extracted from IE1FB2 or IE1TPA15 cells at various times postinfection by the Hirt method (19), and ethanol precipitates were redissolved in TE and quantitated by spectrophotometry. Equal amounts of DNA were digested with *Eco*RI, *Hin*dIII, *Hpa*II, or *Msp*I, the digests were resolved on 0.8% agarose gels, and Southern blotting was done as described previously (41). The blots were probed with a 625-bp *Hin*cII fragment of pIE1FB (23) or a 2-kb *Bam*HI fragment containing the entire t-PA coding sequence, which had been gel purified twice and uniformly labeled by the random-primer method (15). Hybridization was done at 42°C in the presence of 50% (vol/vol) formamide.

RESULTS

Effect of baculovirus infection on production of human t-PA. IE1TPA15 is a stably transformed insect cell line that expresses human t-PA constitutively under the transcriptional control of the IE1 promoter (23). These cells secrete t-PA faster and more efficiently than Sf9 cells infected with a recombinant baculovirus, which is consistent with the idea that the cellular secretory pathway is functionally compromised during baculovirus infection (23, 25). The initial goal of this study was to determine how baculovirus infection would influence the ability of these stably transformed cells to secrete t-PA. If the virus really has an adverse effect on secretory pathway function, then viral infection should inhibit t-PA secretion by these cells.

IE1TPA15 cells from a single large pool were seeded into multiple flasks, mock infected or infected with Ac*M*NPV, and radiolabeled at various times after infection. Extracellular and intracellular fractions were prepared, t-PA was immunoprecipitated and gel purified, and the t-PA bands were quantitated to determine how much radiolabeled product was synthesized and secreted at different times after infection. The results of this analysis showed that there was a steady increase in the amount of extracellular t-PA produced by the mock-infected cells, presumably because they continued to divide as the experiment progressed (Fig. 1). By contrast, baculovirus infection induced a transient increase in extracellular t-PA production from 4 to 8 h postinfection, followed by a sharp decrease to nearly undetectable levels by about 32 h postinfection. Subsequent immunoprecipitation and Western blotting (immunoblotting) analyses failed to reveal any t-PA-related proteins in the intracellular fraction, indicating that a nonsecreted t-PA precursor did not accumulate inside the cells during viral infection (data not shown). These results were consistent with the interpretation that t-PA secretion was blocked and the intracellular precursor was degraded at later times of Ac*M*NPV infection. Alternatively, it was possible that t-PA biosynthesis was blocked at the translational or transcriptional level at late times of infection. Additional experiments were designed to distinguish between these two different possibilities.

Effect of baculovirus infection on production of *E. coli* β-gal. The first approach was to determine how Ac*M*NPV infection would influence the production of a nonsecreted foreign protein by transformed insect cells. If the virus-induced shutdown of t-PA production by IE1TPA15 cells resulted from an adverse effect on secretion, per se, then the production of a nonsecreted foreign protein should not be influenced by baculovirus infection. However, if t-PA expression was blocked at an earlier level, such as transcription or translation, then the expression of a nonsecreted protein should be blocked as well. This analysis was accomplished with another stably transformed insect cell line, IE1FB2, which expresses intracellular β-gal under IE1 control (23). IE1FB2 cells were infected with Ac*M*NPV, and β-gal was extracted from the intracellular fraction and quantitated at various times after infection. Again, there was a transient increase in production of the foreign protein between 4 and 8 h postinfection followed by a sharp decrease to nearly undetectable levels by about 28 h postinfection (Fig. 2). Thus, Ac*M*NPV had nearly identical effects on the production of both secreted and nonsecreted proteins by stably transformed insect cells. This strongly suggested that these effects occurred at the transcriptional or translational level, independently of any viral effects on the cellular secretory pathway.

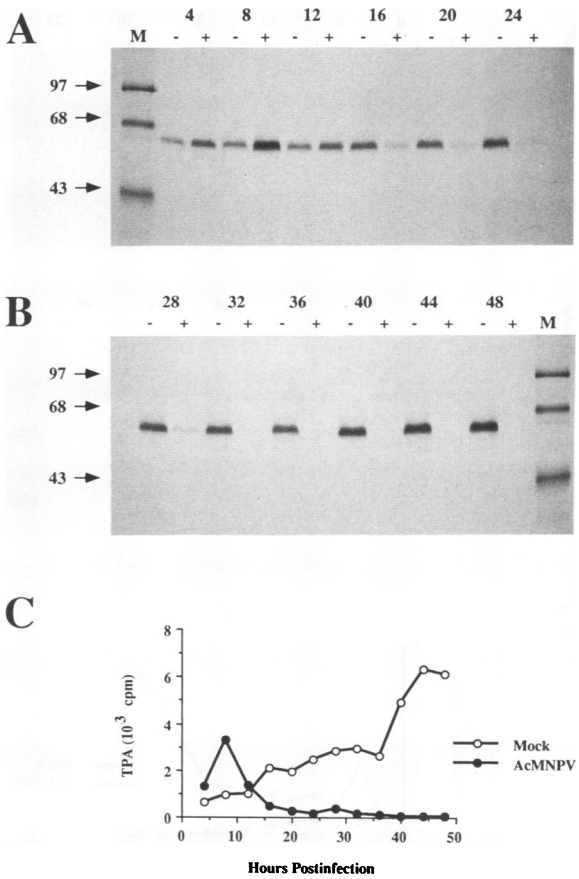

FIG. 1. Effect of baculovirus infection on IE1-mediated t-PA production by transformed insect cells. (A and B) IE1TPA15 cells were mock infected (−) or infected (+) with Ac*M*NPV. At various times after infection, the cells were radiolabeled for 4 h, ending at the times (in hours) indicated above each pair of lanes, and extracellular fractions were harvested. t-PA was immunoprecipitated from the extracellular fractions and analyzed by SDS-PAGE on 12% acrylamide gels. The arrows and numbers to the left of the gels indicate the positions of molecular mass standards (M) and their molecular masses (in kilodaltons). (C) t-PA bands were excised from the gels shown in panels A and B, and radioactivity was quantitated and plotted against time postinfection.

Effect of baculovirus infection on transcription from the integrated IE1 promoter. Quantitative S1 nuclease experiments were done to determine directly whether Ac*M*NPV infection influenced the steady-state levels of IE1-specific transcripts from the integrated IE1TPA or IE1βgal genes in

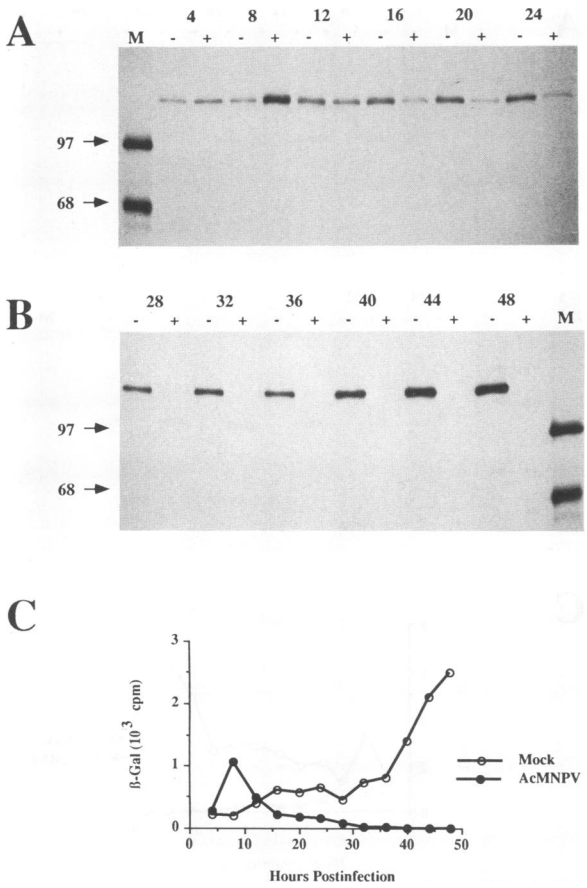

FIG. 2. Effect of baculovirus infection on IE1-mediated β-gal production by transformed insect cells. (A and B) IE1FB2 cells were mock infected (−) or infected (+) with Ac*M*NPV and radiolabeled for 4 h, ending at the times (in hours) indicated above each pair of lanes. Intracellular fractions were prepared, and β-gal was immunoprecipitated and analyzed by SDS-PAGE on 8% acrylamide gels. The arrows and numbers to the left of the gels indicate the positions of molecular mass standards (M) and their molecular masses (in kilodaltons). (C) β-Gal bands were excised from the gels shown in panels A and B and radioactivity was quantitated and plotted against time postinfection.

transformed insect cells. Total cellular RNA was isolated from IE1TPA15 or IE1FB2 cells at various times after mock or Ac*M*NPV infection, and S1 nuclease protection assays were done as described in Materials and Methods. Preliminary experiments established that this assay was both specific and quantitative, because RNA from uninfected IE1TPA15 and IE1FB2 cells protected single fragments of the expected sizes (203 and 290 bp, respectively) and increasing amounts of protection were observed with increasing amounts of total cellular RNA (Fig. 3A). RNA isolated from IE1TPA15 (Fig. 3B) or IE1FB2 (Fig. 3C) cells at various times after mock infection provided essentially equivalent amounts of protection, with no reproducible relationship between the time of mock infection and the amount of probe protected. Conversely, RNA from Ac*M*NPV-infected transformed cells reproducibly provided the highest levels of protection at 4 h postinfection, followed by a steady decline to essentially undetectable levels by 24 h postinfection (Fig. 3B and C, Ac*M*NPV). These results indicated that the steady-state levels of RNA from the

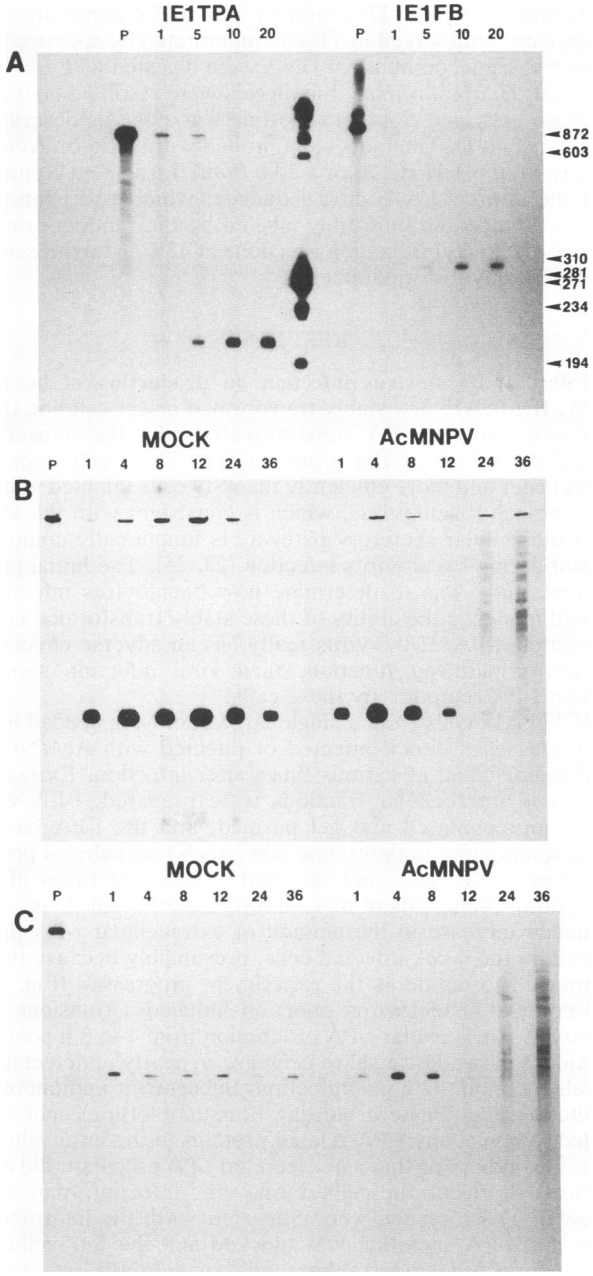

FIG. 3. Effect of baculovirus infection on IE1-mediated foreign gene transcription in transformed insect cells. (A) Total RNA was isolated from uninfected IE1TPA15 or IE1FB2 cells and 1, 5, 10, or 20 μg was used for S1 nuclease assays with t-PA or β-gal probe, respectively. The arrows and numbers to the right of the gel indicate the positions and sizes (in base pairs) of ϕX174-*Hae*III standards. (B) Total RNA was isolated from IE1TPA15 cells at various times after mock or Ac*M*NPV infection and used for S1 nuclease assays with a t-PA probe. (C) Total RNA was isolated from IE1FB2 cells at various times after mock or Ac*M*NPV infection and used for S1 nuclease assays with a β-gal probe. The numbers above the lanes indicate the time (in hours) after mock or Ac*M*NPV infection at which RNA was harvested, while the lanes marked P contained undigested probes. The probes used for these assays are described in detail in Materials and Methods.

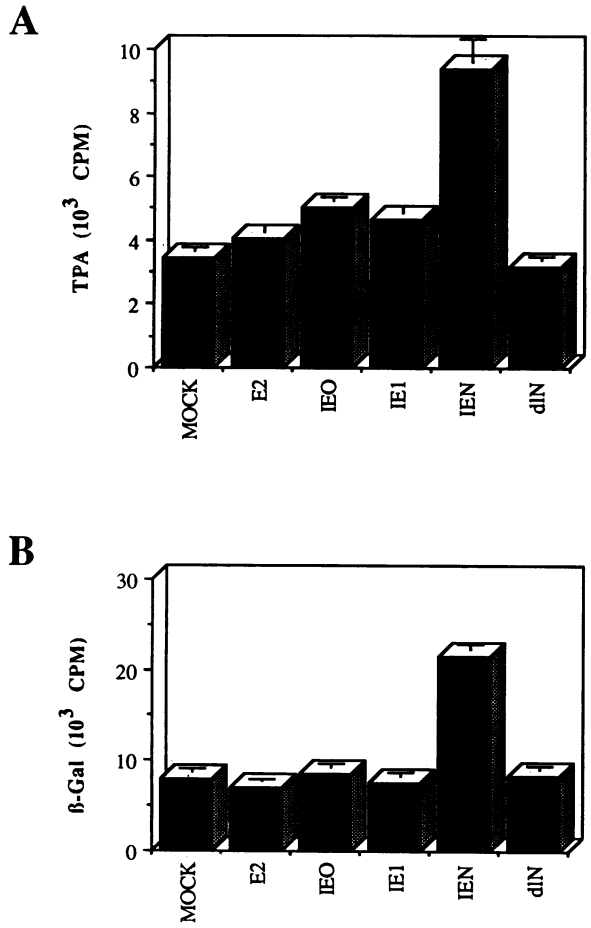

FIG. 4. Effect of immediate-early genes on IE1-mediated foreign gene expression in transformed insect cells. IE1TPA15 (A) or IE1FB2 (B) cells were transfected with 5 μg of calf thymus (MOCK), Ac*M*NPV (E2), or the indicated plasmid DNAs as described in Materials and Methods. At 20 h posttransfection, the cells were radiolabeled for 4 h and extracellular or intracellular fractions were prepared. t-PA and β-gal were immunoprecipitated, resolved by SDS-PAGE, and excised from the gels, and radioactivity was quantitated as described in Materials and Methods.

integrated foreign genes in transformed insect cells increased transiently and then decreased sharply during Ac*M*NPV infection. The disappearance of the single, appropriately sized protected fragment at 24 and 48 h postinfection was accompanied by the appearance of multiple larger fragments; however, if they are specific, the origin of these larger fragments is unknown.

Effect of immediate-early baculovirus genes on IE1-mediated foreign gene expression. It seemed likely that the transient stimulation of transcription from the integrated IE1 promoter during Ac*M*NPV infection resulted from transactivation by immediate-early viral genes. This possibility was tested by transfecting the transformed insect cell clones with plasmids capable of expressing individual immediate-early Ac*M*NPV genes, including IE1, IE0, and IEN. The cells were radiolabeled for 4 h at 24 h after transfection, and the amounts of extracellular t-PA or intracellular β-gal were quantitated. The results of this analysis showed that IEN reproducibly stimulated the expression of either t-PA (Fig. 4A) or β-gal (Fig. 4B). t-PA expression was stimulated by an average of about 2.2-fold (±0.2), and β-gal expression was stimulated by an average of about 2.1-fold (±0.2), as determined in four and five independent experiments, respectively. By contrast, there was no reproducible effect on the expression of these genes by the plasmids encoding IE0, IE1, or a truncated form of IEN. These results suggested that IEN can account for the transient stimulation of the integrated IE1 promoter, which is consistent with the previous conclusion that IEN can transactivate the expression of IE1 and other immediate-early viral genes during Ac*M*NPV infection (5). Although viral DNA also contained the IEN gene, its inability to detectably transactivate the integrated IE1 promoters is probably due to the fact that it provided a much lower gene dosage than an equal amount of the IEN-encoding plasmid. It is harder to explain the inability of IE1 to transactivate the integrated promoters, because previous studies have shown that Ac*M*NPV IE1 can transactivate its own expression in both infected cells and transient assays (31). Perhaps the differences in the local environments of the integrated and viral IE1 promoters preclude the transactivating function of IE1 but not IEN.

Transcriptional activity of the IE1 promoter in the context of the viral or cellular genome. The decrease in the steady-state levels of RNA produced by the integrated IE1 promoter in baculovirus-infected transformed insect cells was surprising, because previous studies have shown that native viral IE1 RNA can be detected throughout Ac*M*NPV infection, reaching peak levels around 24 h (8, 18, 30, 31). The difference in these results suggested that the transcriptional activity of the IE1 promoter at late times of Ac*M*NPV infection depended on its environmental context: while the IE1 promoter in the viral genome remained active, the same promoter in the cellular genome was transcriptionally inactivated. However, this interpretation was based on the assumption that steady-state levels of viral IE1 RNA are maintained at high levels by active transcription from the IE1 promoter throughout infection.

To test this assumption, we measured the half-life of native viral IE1 RNA in baculovirus-infected cells. Sf9 cells were infected with Ac*M*NPV, actinomycin D or ethanol was added at 4 h postinfection, and total RNA was isolated at various times after treatment and subjected to S1 nuclease analysis. Preliminary experiments established that [^{3}H]UdR incorporation was inhibited by 96 and 98% after 0.5 and 1 h of actinomycin D treatment, respectively, which indicated that total RNA synthesis was effectively blocked (data not shown). RNA isolated from control, ethanol-treated cells protected a 341-bp fragment, as expected for transcripts initiating at the CAGT motif in the IE1 promoter, and a 531-bp fragment, as expected for transcripts initiating at the CAGT motif in the IE0 promoter (Fig. 5A [8, 31]). Essentially equivalent amounts of the 341-bp IE1-specific fragment were protected after various times of ethanol treatment, while decreasing amounts of this fragment were protected with increasing length of actinomycin D treatment. Direct quantitation of the 341-bp fragment after various times of actinomycin D treatment revealed that the half-life of native IE1 RNA was approximately 3 h in baculovirus-infected cells. Thus, these results suggest that high steady-state levels of viral IE1 RNA are maintained by active transcription from the viral IE1 promoter throughout infection. It follows that the decreased levels of steady-state RNA from the integrated genes in transformed cells infected with Ac*M*NPV result from position-dependent inactivation of transcription from the integrated IE1 promoter. This indicates that the same transcriptional promoter can behave in dra-

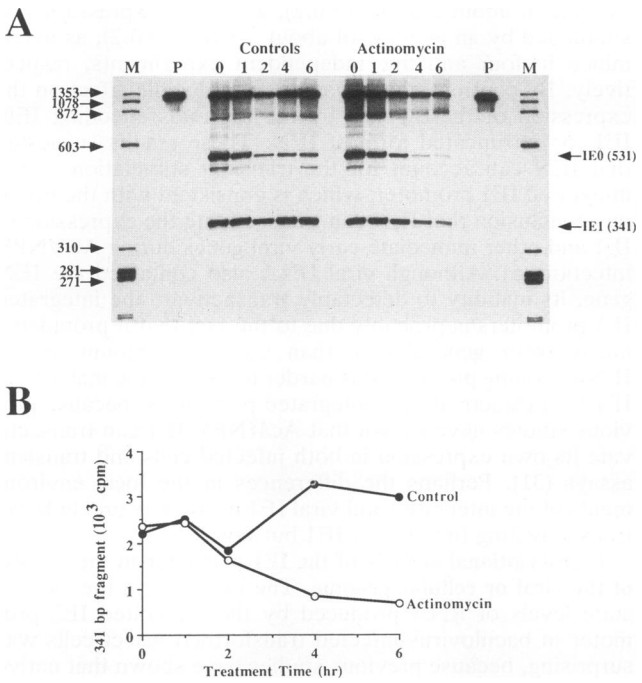

FIG. 5. Stability of IE1 RNA in baculovirus-infected cells. (A) Ethanol (controls) or actinomycin D was added to Ac*M*NPV-infected Sf9 cells at 4 h postinfection. At various times after treatment, total RNA was isolated and used for S1 nuclease assays with an IE1/IE0 probe as described in Materials and Methods. The numbers shown above the lanes indicate when (in hours) the RNA was harvested after treatment. The lanes marked P contained undigested probe, while the lanes marked M contained ϕX174-*Hae*III markers. The positions and sizes (in base pairs) of the markers are indicated on the left, while the positions and sizes of the IE1- and IE0-specific protected fragments are indicated on the right. (B) The 341-bp protected fragments in the gel shown in panel A were quantitated by direct scanning for radioactivity with a Betascope model 603 blot analyzer (Betagen, Waltham, Mass.), and the results were plotted against time after ethanol or actinomycin D treatment.

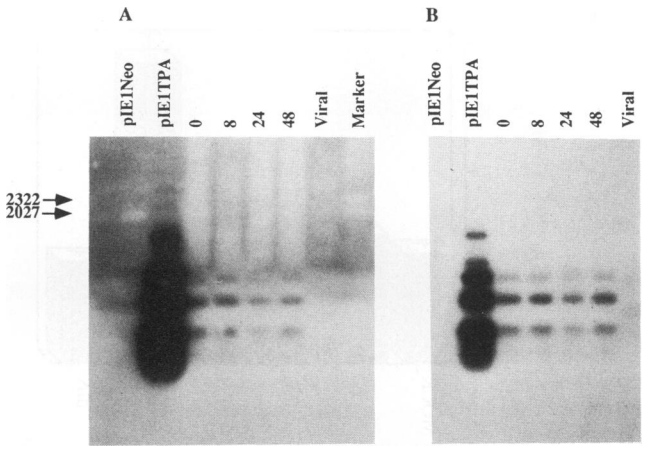

FIG. 6. Methylation of integrated foreign gene sequences during Ac*M*NPV infection. High-molecular-weight DNA was isolated from IE1TPA15 cells at various times after Ac*M*NPV infection and digested with *Hpa*II (A) or *Msp*I (B), and the digests were resolved on 0.8% agarose gels. Southern blots were prepared and hybridized with a t-PA probe, as described in Materials and Methods. The labels above the lanes indicate plasmid controls (pIE1Neo and pIE1TPA), a viral DNA control (viral), or the time (in hours) after infection the high-molecular-weight DNA was isolated. The positions of λ-*Hin*dIII markers are shown on the left in base pairs.

matically different ways at late times of baculovirus infection, depending upon its environmental context.

Effects of baculovirus infection on the host genome. Theoretically, the inhibition of transcription from the integrated IE1 promoter during baculovirus infection could result from virus-induced methylation of the host cell genome. Cytosine methylation at the dinucleotide sequence CpG is associated with transcriptional inactivation of eucaryotic genes (14). It is generally thought that insect cell DNA, including Sf9 cell DNA, contains little, if any, 5-methylcytosine (28). However, the possibility that Ac*M*NPV could induce methylation of the host cell genome has not been addressed. The t-PA coding sequences in IE1TPA15 cells contain multiple sites for the restriction endonucleases *Hpa*II and *Msp*I, which both recognize the sequence 5′-CCGG-3′. Cleavage by *Hpa*II, but not *Msp*I, is blocked by methylation of the internal cytosine residue. Thus, cytosine methylation of the integrated sequences would be revealed by differences in the Southern blotting patterns obtained with these two enzymes. Genomic DNA was isolated from IE1TPA15 cells at various times after Ac*M*NPV infection and then digested with *Hpa*II or *Msp*I, and Southern blots were prepared and probed with a 2-kb *Bam*HI fragment containing the entire t-PA coding sequence. The resulting hybridization patterns were identical whether the DNA was digested with *Hpa*II or *Msp*I, and there was no change in the patterns at different times of Ac*M*NPV infection (Fig. 6). These results suggested that the integrated t-PA gene is not methylated at CpG and, therefore, that cytosine methylation does not account for its transcriptional inactivation at late times of baculovirus infection.

Wilson and Miller (46) have previously shown that there are no major changes in the structure of host chromatin during baculovirus infection. Moreover, Ac*M*NPV actually encodes a gene that inhibits the endonucleolytic breakdown of chromatin, which occurs during the process of programmed cell death, or apoptosis (9). However, it was formally possible that the integrated foreign genes in the transformed cell lines were somehow destabilized, excised, and degraded during Ac*M*NPV infection, which would explain why they were not expressed at late times of infection. To address this possibility, we isolated genomic DNA from IE1FB2 cells at various times after infection and performed Southern blotting analyses. The genomic DNA was digested with *Hin*dIII, which cuts the integrated pIE1FB plasmid once (23), and the blots were hybridized with a β-gal-specific probe. The results showed that the integrated β-gal sequences were stable from 0 to 72 h postinfection (Fig. 7). Thus, virus-induced destabilization and degradation of the integrated foreign gene sequences do not account for the transcriptional inactivity of the integrated IE1 promoter at late times of Ac*M*NPV infection.

DISCUSSION

Previously, we suggested that the cellular secretory pathway is functionally impaired during baculovirus infection, on the basis of the observation that the efficiency of t-PA secretion decreased with increasing time of recombinant baculovirus infection (25). In support of this idea, we produced transformed insect cells that express t-PA in the

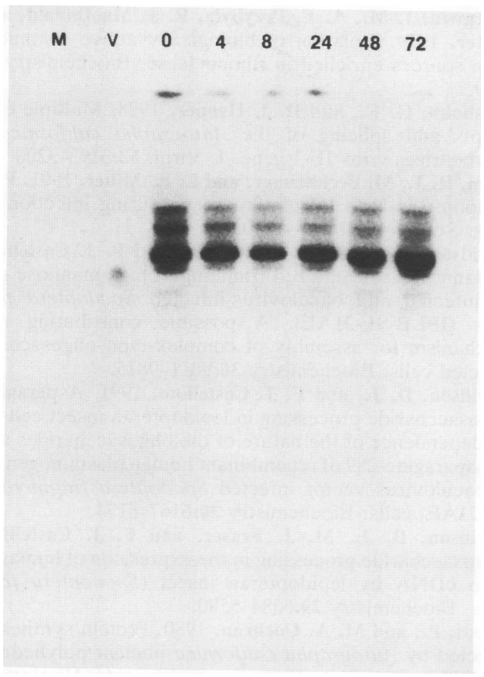

FIG. 7. Stability of integrated foreign gene sequences during Ac*M*NPV infection. High-molecular-weight DNA was isolated from IE1FB2 cells at various times after Ac*M*NPV infection and digested with *Hin*dIII, and the digests were resolved on 0.8% agarose gels. Southern blots were prepared and hybridized with a β-gal probe, as described in Materials and Methods. The labels above the lanes indicate that the viral DNA control (V) was used or the time (in hours) after infection the high-molecular-weight DNA was isolated.

absence of viral infection and showed that they secreted this foreign gene product more efficiently and quickly than infected insect cells (23). The obvious extension of this work was to determine how baculovirus infection would affect the ability of the transformed cells to secrete t-PA. If virus infection compromises the function of the cellular secretory pathway, then the ability of these cells to secrete t-PA should be reduced or eliminated.

As expected, Ac*M*NPV blocked the production of extracellular t-PA by transformed insect cells. However, further analysis revealed that this did not necessarily reflect an effect of virus infection on the function of the cellular secretory pathway. Ac*M*NPV also blocked the ability of a transformed cell line to produce an intracellular protein, β-gal. This suggested that Ac*M*NPV might block expression of these products by transformed insect cells at the transcriptional or translational level. The results of quantitative S1 nuclease assays showed directly that expression was blocked at the transcriptional level. Thus, this study did not provide further evidence that the host cell secretory pathway is functionally compromised during Ac*M*NPV infection. However, this study did not provide any evidence to the contrary, either. Viral effects on the secretory pathway would have gone undetected because only t-PA and β-gal production were examined, and both were blocked at the transcriptional level.

The finding that Ac*M*NPV infection blocks transcription of the integrated foreign genes in transformed insect cells was quite surprising. Transcription of the integrated genes in these cells is controlled by the viral IE1 promoter, and previous studies had shown that the steady-state levels of native viral IE1 RNA remain high throughout infection (8, 18, 30, 31). The native IE1 RNA pool might result from the accumulation of highly stable transcripts produced early in infection or it might be maintained by continuous transcription from the IE1 promoter throughout infection. The finding that native IE1 RNA has a half-life of only about 3 h supported the latter possibility. This suggested, in turn, that the reduction in steady-state levels of RNA from the integrated foreign genes in transformed insect cells at late times of baculovirus infection probably results from transcriptional inactivation of the integrated IE1 promoter. The finding that the same promoter can remain active in the context of the viral but not the cellular genome shows that transcriptional activity can be strongly influenced by the local environment of a promoter during the late phases of Ac*M*NPV infection. This conclusion is consistent with the previous finding that a consensus baculovirus late promoter in a lepidopteran insect cell retrotransposon is active in the context of the viral but not the cellular genome at late times of infection (16).

The effects of baculovirus infection on host gene expression have been examined in several studies. It is known that host cell protein synthesis is blocked (6, 13, 27) and that transcription of at least some cellular genes is significantly reduced (38) at late times of Ac*M*NPV infection. On the basis of these findings, a reasonable hypothesis is that Ac*M*NPV shuts down host gene expression by inhibiting host protein synthesis, which is accompanied by the turnover of labile transcription factors required for cellular gene expression. As the infection progresses, these transcription factors are no longer needed for the transcription of viral genes, which is mediated by virus-encoded factors and can occur in the absence of cellular gene expression. Within the confines of this simple model, however, it is difficult to understand how the IE1 promoter in the viral genome can remain active throughout Ac*M*NPV infection and why this same promoter in the cellular genome is only active during the early phases of infection. The former observation could be explained if the new transcription factors can initiate transcription from the CAGT motif in the viral IE1 promoter during the late phase of infection. However, this model cannot explain why these putative factors cannot initiate transcription from the integrated IE1 promoter as well. Perhaps some other virus-induced event selectively inactivates the integrated form of the IE1 promoter. This might be a general effect on host chromatin that inhibits the expression of all (?) cellular genes at the transcriptional level, irrespective of their promoters. However, this dramatic effect would have to result from a rather subtle alteration, because Wilson and Miller (46) have shown that there are no detectable changes in the nucleosomal structure of host chromatin and we have shown that there is no cytosine methylation of host cell DNA or degradation of the integrated genes. Perhaps the redistribution of cellular chromatin to the periphery of the nucleus, termed margination in some ultrastructural studies (1), results in the transcriptional inactivation of cellular promoters. Alternatively, host gene expression might be shut down posttranscriptionally, by virus-induced destabilization of RNA at late times of infection, as originally proposed by Ooi and Miller (38). The simplest form of this model requires some universal difference between host and late viral RNAs and the common sequence (AAG) found on the 5′ ends of all late viral RNAs might be the distinguishing feature that allows them to survive (38). However, the 5′ ends of the transcripts pro-

duced by the integrated and viral IE1 promoters are probably identical, and neither begins with AAG. Therefore, differences in their 5′ ends cannot explain how the cellular IE1 transcripts are preferentially destabilized, and the 5′ AAG sequence cannot be the universal signal for selective RNA destabilization. Perhaps there is something unique about the 3′ end of viral IE1 RNA that allows it to survive during the late phases of baculovirus infection. Obviously, further work is required to test these speculations and to more clearly elucidate the effects of baculovirus infection on host gene expression.

One potential application of transformed insect cells is as genetically modified hosts that can be used for the production of certain types of foreign gene products by baculovirus expression vectors. For example, transformed insect cell lines could be made to express the genes encoding mammalian N-linked oligosaccharide-processing enzymes, some of which appear to be missing in insect cells (2, 3, 20, 29, 32), and these cells could be used to produce glycoproteins with more highly processed glycans in the baculovirus expression vector system. However, the results of this study indicate that this will not be straightforward. IE1-mediated expression of the integrated genes in transformed insect cells is likely to be blocked at the transcriptional level at late times of baculovirus infection, when the foreign gene of interest is just beginning to be expressed. Thus, transformed insect cells could only serve as modified hosts for baculovirus expression vectors if the products of the integrated foreign genes have long half-lives and can remain functional after transcription is shut down by the virus infection. Another potential application of transformed insect cells, as helper cell lines for the propagation of Ac*M*NPV mutants in essential genes, is feasible for mutants in early viral functions. However, the use of this approach for mutants in essential late functions will depend upon the half-lives of the integrated gene products, as described above.

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