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Promoter Influence on Baculovirus-Mediated Gene Expression in Permissive and Nonpermissive Insect Cell Lines

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The activities of viral and insect promoters were examined in a range of insect cell lines permissive and nonpermissive for the replication of the baculovirus *Autographa californica* nuclear polyhedrosis virus. Recombinant baculoviruses were constructed to place the bacterial chloramphenicol acetyltransferase gene under the control of promoters strongly active in the early, late, or very late stages of virus replication. In fully permissive cells, expression from a very late promoter was 2- to 3-fold higher than expression from a late promoter and 10- to 20-fold higher than expression from an early promoter or from a virus-borne insect promoter. In cell lines that do not support the efficient production of viral progeny, late-promoter-driven expression was similar to or surpassed very late promoter-driven expression. In nonpermissive insect cell lines, expression driven by an insect promoter derived from *Drosophila melanogaster* was higher than expression from the three viral promoters and was especially high in the *Drosophila* cell line tested. Surprisingly, late-promoter-driven expression, which is dependent on DNA replication, was higher than early-promoter-driven expression in three of four nonpermissive lines. In contrast, very late promoter-driven expression was quite limited in nonpermissive cell lines. The results indicate that the promoter used to drive foreign-gene expression strongly influences the range of insect cells which can efficiently support the production of the foreign protein during infection with recombinant baculoviruses.

Three phases of transcriptional activation—early, late, and very late—can be distinguished during the replication cycle of the baculovirus *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV). Early viral transcripts are synthesized by an α-amanitin-sensitive RNA polymerase (10, 21), presumably host RNA polymerase II. This view is supported by the observation that early viral genes are transcribed in the presence of inhibitors of protein synthesis (9, 37, 50) and in nuclear extracts from uninfected cells (19), although it is possible that virion structural proteins also contribute to early viral gene transcription. Early-gene products include enzymes involved in viral DNA replication (9, 27, 50) and factors which can modulate early-gene expression (5, 11, 24). Activation of late- and very late gene transcription is dependent on both early-gene expression and DNA replication (20, 21, 42, 45) and involves the induction of a virus-induced RNA polymerase activity that is insensitive to α-amanitin (10, 21). Late-gene products provide the structural proteins and other products necessary for budded virus production (45, 52) and appear to be involved in activating very late gene transcription (35). Very late genes furnish proteins for the production of occluded virus at very late times in infection (18, 26).

An analysis of the ability of promoters from each temporal transcription class to drive gene expression in a range of insect cell lines is of interest from several different perspectives. Carbonell et al. (2) showed that Ac*M*NPV enters a wider range of insect cells than can be productively infected and expresses a reporter gene if it is under the control of an RNA polymerase II-recognized promoter but not a viral very late promoter (VLP). Thus, the range of insect cells which can be productively infected by baculoviruses may be limited by deficiencies in either the timing or the extent of gene expression. A comparison of the strength and timing of expression from different viral promoters would provide information about the point at which a productive infection is blocked in refractive cell lines.

The relative activity of early (EP), late (LP), and very late (VLP) virus promoters is also of interest from the perspective of baculovirus expression systems. The expression of foreign genes from baculovirus vectors can yield high levels of protein for commercial or research purposes. Although the standard procedure for producing a protein from recombinant baculoviruses is to place the gene of interest under the transcriptional control of a VLP, it may be desirable to produce a protein earlier in infection, before cellular processes involved in posttranslational modification and secretion are compromised by the progress of viral infection (22, 36). This may be accomplished by using an EP or LP to drive expression (16, 38, 45). A systematic comparison of the kinetics and levels of expression from promoters belonging to the three different promoter classes has never been reported and is clearly important in assessing the relative advantages of the different promoters for expression vector purposes.

Another application of promoter analysis in permissive and nonpermissive cells is in assessing the safety and improving the efficacy of baculoviruses as biological control agents for insect pests. Baculoviruses have been engineered genetically to produce foreign proteins, such as insect-selective toxins, so that pest insects are quickly incapacitated after infection (29, 30, 32, 44, 48, 49). The promoter chosen to drive foreign gene expression may affect the range of insects incapacitated by the recombinant viral pesticide as well as determine the timing and overall level of expression of the insecticidal gene. Carbonell et al. have shown that while mammalian cells are refractive to Ac*M*NPV infection (3), the virus is able to enter cells of taxonomically diverse insects and to express a chloramphenicol acetyltransferase

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(CAT) gene under the control of the Rous sarcoma virus long terminal repeat promoter (2). In contrast, the expression of a marker gene controlled by the very late polyhedrin promoter was detected only in permissive cells (2). Thus, the range of insects which might be affected by toxin-expressing recombinant baculoviruses may not be limited by entry into cells but by the promoter used to drive toxin gene transcription. Promoter effects are therefore important from the perspective of both environmental safety and pesticide design.

We have examined the temporal pattern and the relative strength of expression from three viral promoters representing the early, late, and very late promoter classes and an insect promoter, a *Drosophila* hsp70 promoter (HP), during recombinant virus infection of seven cell lines representing two insect orders and four lepidopteran families. Our data indicate that VLP activity is limited primarily to permissive cells. In nonpermissive cells, expression from an LP is not as high as in permissive cells but is higher than expression from viral EPs. Levels of expression in nonpermissive cells from the virus-borne HP are higher than from any of the viral promoters and are especially high in *Drosophila* cells. The implications of our findings for baculovirus host range, foreign gene expression, and biopesticide development and safety are discussed.

MATERIALS AND METHODS

Cells and media. *Spodoptera frugiperda* (fall armyworm) IPLB-SF21 (SF21) cells (51) and *Bombyx mori* (silkworm) BmN-4 cells (28) were maintained in TC100 medium (GIBCO BRL, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (GIBCO BRL) and 0.25% tryptose broth (Difco, Detroit, Mich.). *Helicoverpa zea* (cotton bollworm) Hz1b3 cells (41) and *Lymantria dispar* (gypsy moth) IPLB-Ld652Y (Ld652Y) cells (12) were maintained in the serum-free medium SF900 (GIBCO BRL). *Choristoneura fumiferana* (spruce budworm) IPRL-CF-1 (CF-1) cells (17) were maintained in TMN-FH medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 10% fetal bovine serum (GIBCO BRL). *Mamestra brassicae* (cabbage armyworm) SES-MaBr-3 (MaBr-3) cells (17) were maintained in Mitsuhashi and Maramorosch medium (Sigma) supplemented with 10% fetal bovine serum (GIBCO BRL). *Drosophila melanogaster* Schneider 1 (Dm) cells (43) were maintained in Schneider medium (Sigma) supplemented with 15% fetal bovine serum and 5 mg of Bacto Peptone (Sigma) per ml. All cell lines were maintained at 27°C, and all except SF21 cells were grown in T-75 flasks (Corning Glass Works, Corning, N.Y.). SF21 cells were cultured as described previously (33). Hz1b3, CF-1, Ld652Y, and MaBr-3 cells were split approximately 1:5 every 4 days. BmN-4 cells were split 1:2 and Dm cells were split 1:10 every 4 to 5 days.

Recombinant plasmids and viruses. The plasmids phcIE0, phcIE1, phcIEN, phcPE38, phcETL, and phcHSP70PL were constructed by replacing 92 bp between the unique *Eco*RV and *Bgl*II sites of the transplacement vector phcwt (40) with the six different promoters described below. This replacement strategy fuses the promoter and some untranslated mRNA leader sequences from each gene to sequences upstream of the translational initiation codon of the CAT reporter gene. The base pair numbers given below are relative to the transcriptional initiation site of each gene.

The IE0 promoter in phcIE0 consists of 575 bp extending from the *Sna*BI site at −539 to the *Bss*HI site at +36 (8). The IE1 promoter in phcIE1 consists of 560 bp extending from the *Cla*I site at −550 to the *Hin*cII site at +10 (14). The IEN promoter in phcIEN includes sequences from −382 to +38 from the IEN transcriptional start site reported in reference 4. The PE-38 promoter in phcPE38 includes sequences extending from −374 to +49 (25). The ETL promoter in phcETL consists of sequences extending from approximately −280 to +38 (9). The *D. melanogaster* HP in phcHSP70PL includes sequences from approximately −500 to +231 (47).

The recombinant viruses vIE0cat, vIE1cat, vETLcat, and vHSP70PLcat were constructed by standard baculovirus allele replacement techniques (33). The plasmids phcIE0, phcIE1, phcETL, and phcHSP70PL were cotransfected with viral DNA from either Ac*M*NPV L-1 (for vHSP70PL) or a modified Ac*M*NPV L-1 derivative, vDA26Z (39) (for vIE1cat, vIE0cat, and vETLcat). The recombinant viruses vCapcat324 (46) and vhcLSXIV (35) have been described previously.

Viral infections and determination of CAT specific activity. Cell monolayers were infected with a quantity of recombinant virus equivalent to 20 PFU/cell in SF21 cells. Cells were refed with fresh medium after a 1-h adsorption period at room temperature. For aphidicolin treatment, cells were refed with medium containing 5 μg of aphidicolin (Sigma) per ml. At the times indicated for each experiment, cells were harvested and extracts were prepared by the methods of Gorman et al. (13), using three freeze-thaw cycles to disrupt the cells. The total protein content of each extract was determined by the Bio-Rad (Melville, N.Y.) protein miniassay procedure, which is based on the method of Bradford (1). The CAT specific activity of each extract was calculated and expressed as nanomoles of [^{14}C]chloramphenicol acetylated per minute per milligram of protein.

Primer extension analysis. Total RNA was isolated from infected cells by the method of Chirgwin et al. (7). A 15-μg sample of each RNA was combined with 1.5 pmol of 5′-end-labeled CAT-specific primer (40), denatured at 90°C, and annealed for 3 h at 45°C in hybridization buffer (400 mM NaCl, 40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.4], 1 mM EDTA). Samples were put on ice, diluted 10-fold in reverse transcription buffer (50 mM Tris-HCl [pH 8.3], 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, 50 ng of actinomycin D per μl, 0.5 mM each deoxynucleoside triphosphate). After the addition of 200 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL), the reaction mixes were incubated at 37°C for 1 h, extracted, precipitated, and analyzed on denaturing 8% polyacrylamide–7 M urea sequencing gels. The same labeled primer was used to prime denatured plasmid DNA in chain-terminating sequencing reactions (6).

RESULTS

Experimental design. We have constructed a series of recombinant viruses to examine promoter-dependent expression of a CAT reporter gene in permissive and nonpermissive insect cell lines. Because CAT protein levels reflect the transcriptional activity of a promoter indirectly, we have taken special care to construct promoter-CAT gene fusions that minimize possible differences in the stability or translational efficiency of different CAT mRNAs. In a study with a series of viral VLP-CAT gene fusions, Ooi et al. (35) demonstrated that neither the length nor the specific sequence of nucleotides added to the CAT mRNA leader had a significant effect on CAT mRNA stability. Thus, in the present investigation, viral promoter sequences were fused to the CAT sequences in the 5′ leader region so that the

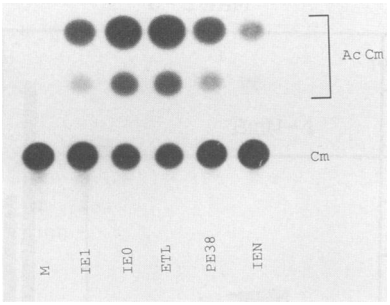


FIG. 1. Analysis of viral EP activity in a transient-expression assay. Each plasmid expressing the CAT gene under the transcriptional control of different viral EPs was transfected (2 μg) separately into cultures of SF21 cells (2×10^6 cells per 60-mm dish). A plasmid encoding the IE1 gene product was also included in each transfection (1.5 μg). The cells were harvested 48 h after transfection and assayed for CAT activity. M, mock-transfected cells; other lanes, cells transfected with plasmids phcIE1, phcIE0, phcETL, phcPE38, or phcIEN, as indicated. Cm, [^{14}C]chloramphenicol; Ac Cm, acetylated [^{14}C]chloramphenicol.

length of the mRNA leader increased by only 10 to 50 nucleotides. To minimize different translation efficiencies, the fusions were made so that all CAT mRNAs contained identical sequences from 37 nucleotides upstream of the AUG start codon through the CAT protein-coding region to the untranslated 3′ terminus.

The *Drosophila* HP-CAT fusion used in the experiments adds 231 nucleotides to the CAT mRNA leader. Sequences in the hsp70 leader are thought to be involved with increased translational efficiency during heat shock in some cell types (23). However, Ac*M*NPV infection does not induce a heat shock response in SF21 cells (8a), and thus it is unlikely that the preferential translation function of these sequences is active during infection.

Comparison of the activities of selected viral EPs. To identify strong EPs that could potentially express high levels of a foreign gene product at early times postinfection (p.i.), we constructed a series of five CAT expression plasmids that are identical except for the different EPs that drive CAT expression. We initially compared the activities of these EPs by transient-expression assays in the permissive SF21 cell line. Each expression plasmid was cotransfected along with a second plasmid encoding the IE1 gene product under the control of the IE1 promoter. In transient-expression assays, the IE1 gene product is necessary and sufficient for strong expression from the ETL promoter (52a) and has been shown to modulate IE1, IE0, and IEN promoter activity (5, 24). The results (Fig. 1) indicate that, in SF21 cells, the ETL and IE0 promoters yield the highest levels of CAT protein in transient-expression assays.

To compare the activities of these promoters during infection, we constructed recombinant viruses carrying the CAT gene under the control of three of the viral EPs tested in transient-expression assays. Permissive SF21 cells and nonpermissive Hz1b3 cells were infected with the recombinant viruses vIE1cat, vIE0cat, and vETLcat. An analysis of the CAT activities of extracts prepared from infected cells at various times p.i. indicates that the ETL promoter yields the highest CAT levels in both the *S. frugiperda* and *H. zea* cell lines (Fig. 2).

Promoter-driven expression in different cell lines. Having determined that the ETL promoter was the strongest of the EPs tested, we chose the recombinant virus vETLcat for further study of viral EP function in a variety of different cell lines. The recombinant virus vCapcat324 (46), which expresses CAT under the control of the vp39 promoter, was chosen to study LP activity. The product of the vp39 gene, the major capsid protein, is a late-expressed protein found in abundance between 10 and 20 h p.i. For an analysis of VLP

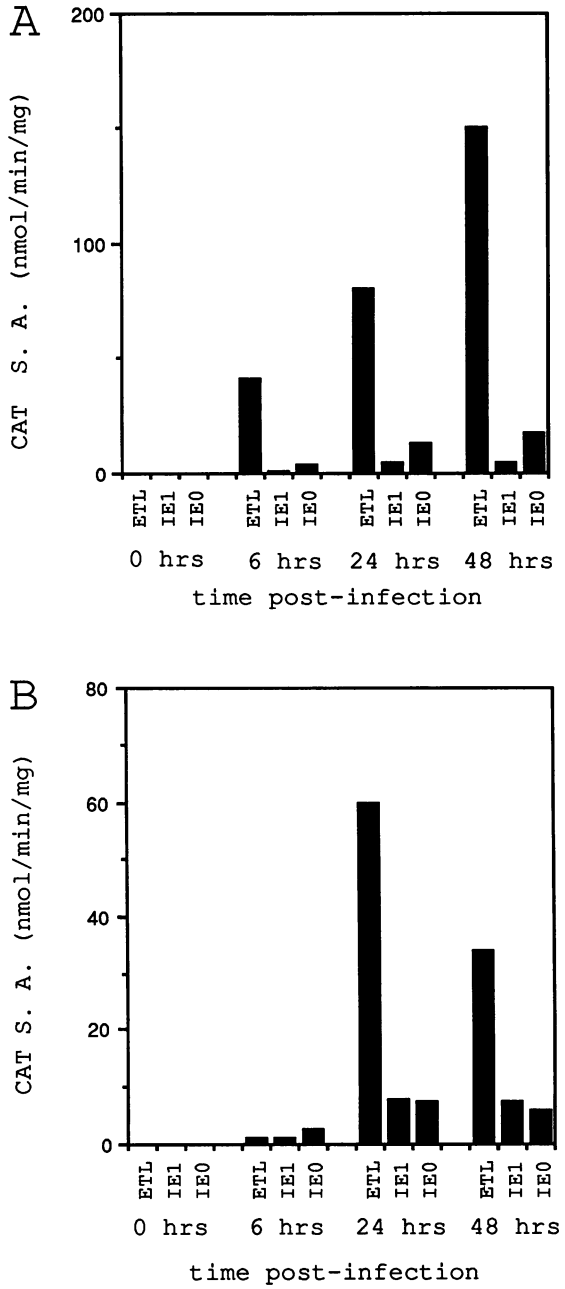
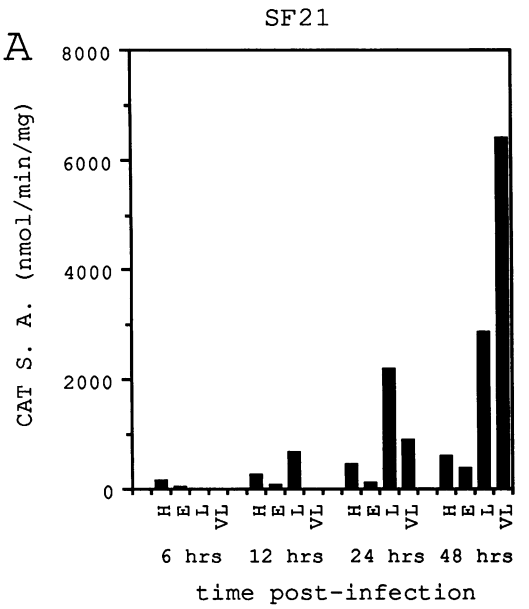
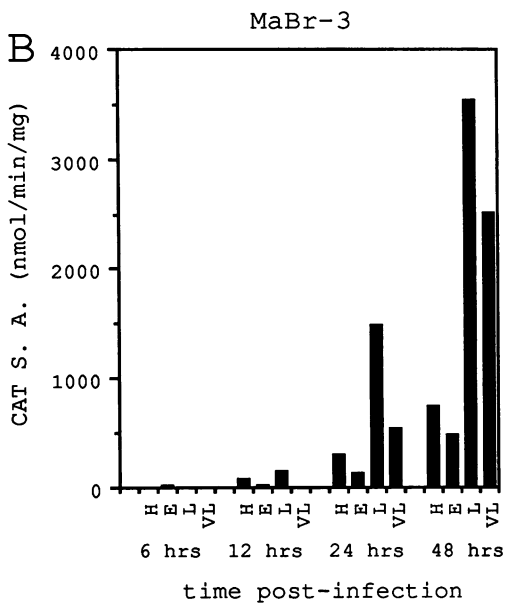


FIG. 2. Analysis of viral EP activity in permissive and nonpermissive cells with recombinant viruses. The SF21 (A) and Hz1b3 (B) cell lines were infected with recombinant viruses vETLcat, vIE1cat, and vIE0cat, which express the CAT gene under the transcriptional control of the ETL, IE1, and IE0 promoters, respectively. The graphs indicate the CAT specific activities (CAT S. A.) of whole-cell extracts isolated from cells harvested at the given times after infection. The zero time point was immediately after the 1-h adsorption period.





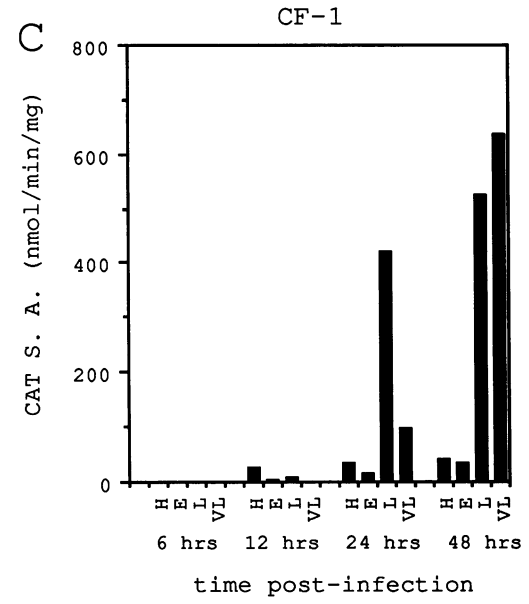


FIG. 3. Analysis of promoter activities during recombinant virus infection of permissive and partially permissive cell lines. The SF21 (A), MaBr-3 (B), and CF-1 (C) cell lines were infected with recombinant viruses expressing CAT under the transcriptional control of, respectively, a *Drosophila* hsp70 promoter (H), the ETL viral EP (E), a viral LP (L), and a viral VLP (VL). The graphs display the CAT specific activity (CAT S. A.) of whole-cell extracts from infected cells harvested at the times indicated.

activity, we used vhcLSXIV, which expresses CAT from a modified version of the polyhedrin promoter. Temporal regulation of the wild-type polyhedrin promoter and the modified LSXIV polyhedrin promoter is identical (35). The LSXIV promoter is more active than the wild-type polyhedrin promoter (35) and thus facilitates the detection of VLP activation in cell lines exposed to the vhcLSXIV virus.

The results of Carbonell et al. (2) suggested that a virus-borne "host" promoter would be active at early times p.i. We have tested this concept with the recombinant virus vHSP70PLcat, which carries a CAT gene under the control of a *Drosophila* hsp70 promoter.

Several different permissive and nonpermissive insect cell lines were infected with each of the four recombinant viruses. An amount of virus stock equivalent to 20 PFU per cell in SF21 cells was applied to each culture plate. Cells were harvested at 6, 12, 24, and 48 h p.i., total cell protein extracts were prepared, and the CAT specific activity of the extracts was determined (Fig. 3 and 4).

SF21 cells are a fully permissive line commonly used for Ac*M*NPV propagation. At 6 h p.i., CAT activity was detected from the HP and from the ETL promoter (EP) (Fig. 3A) but not from the vp39 promoter (LP) or the linker-modified polyhedrin promoter (VLP). CAT levels from the HP are about threefold higher than from the EP. By 12 h p.i., the levels of CAT from the LP have already surpassed the levels from the EP and HP. By 24 h p.i., the VLP becomes active but does not surpass LP production until 48 h p.i. At 48 h p.i., VLP activity has resulted in two- to threefold more product than LP activity.

The MaBr-3 and CF-1 cell lines do not support the efficient production of Ac*M*NPV progeny. Titers of budded virus increase only 10-fold during Ac*M*NPV infection of MaBr-3 cells, and no increase in titer is detected in CF-1 cells (34a). Polyhedral inclusion bodies are observed in 10 and 3% of MaBR-3 and CF-1 cells, respectively (34a). However, levels of expression from the promoters in infected MaBr-3 and CF-1 cell lines follow the same basic pattern as in SF21 cells (Fig. 3B and C). The promoters are expressed in the same temporal order as observed in SF21 cells. Expression from the HP is higher than from the EP, and LP- and VLP-driven expression surpasses HP- and EP-driven expression by 24 h p.i. A major difference among the three cell lines in the first 48 h of infection is that expression from the VLP in MaBr-3 and CF-1 cells relative to LP-driven expression is significantly less than observed in SF21 cells. This suggests that VLP activity is impaired or delayed in these two cell lines,

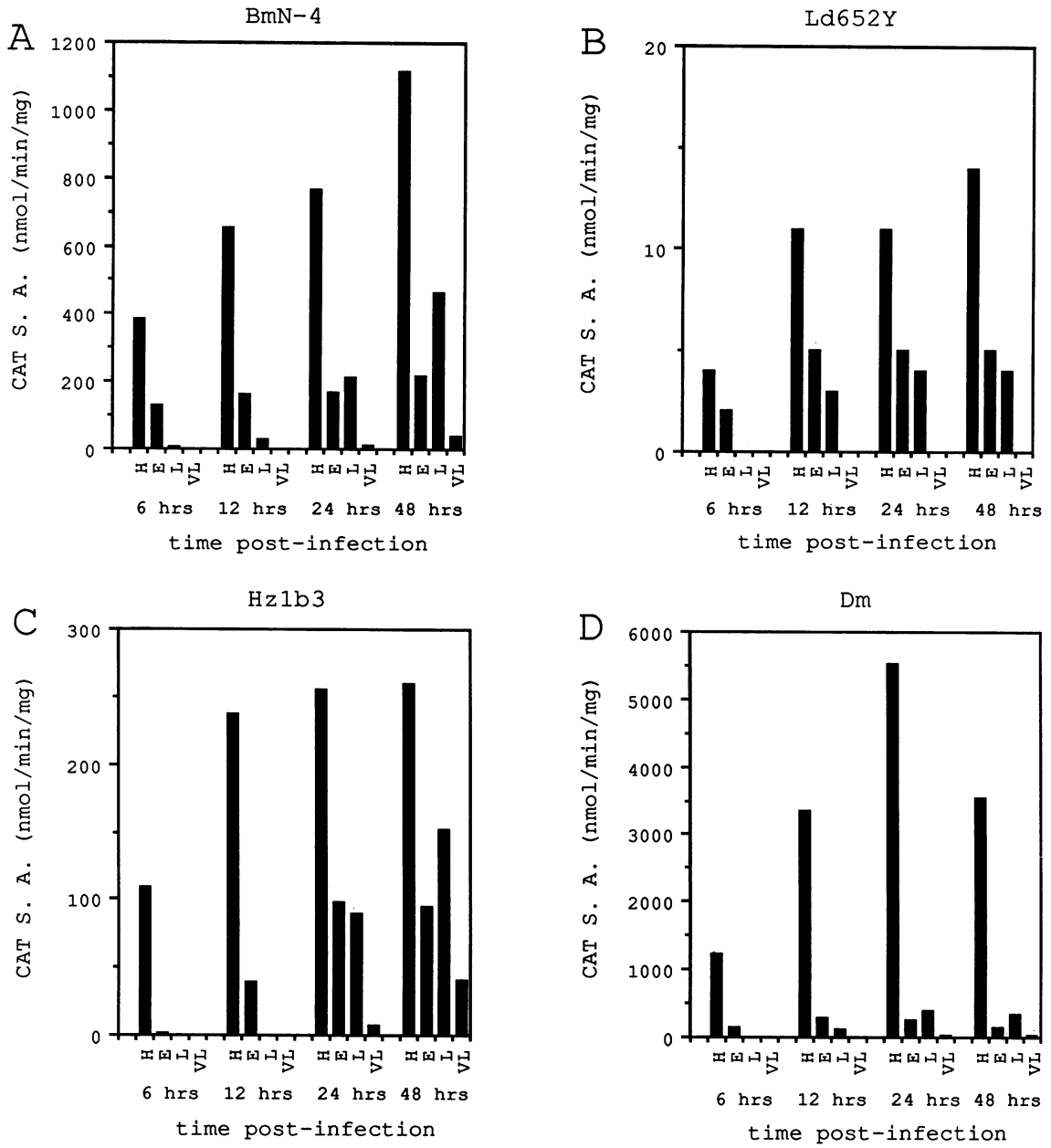


FIG. 4. Analysis of promoter activities during recombinant virus infection of nonpermissive cell lines. The BmN-4 (A), Ld652Y (B), Hz1b3 (C), and Dm (D) cell lines were infected with recombinant viruses expressing CAT under the transcriptional control of a *Drosophila* hsp70 promoter (H), the ETL viral EP (E), a viral LP (L), or a viral VLP (VL). The graphs display the CAT specific activities (CAT S. A.) of whole-cell extracts from infected cells harvested at the times indicated.

although we cannot rule out cell line-specific translational differences from these data alone.

The activity of the promoters was also tested in four cell lines in which no detectable levels of budded virus or polyhedral inclusion bodies are produced by Ac*M*NPV infection (BmN-4, Ld652Y, Hz1b3, and Dm) (34a). Although the same basic order of activation observed in permissive cells is observed in these nonpermissive lines, (Fig. 4), the relative levels of expression from the promoters are quite different than those observed in the more permissive lines (Fig. 3). In each nonpermissive cell line, HP-driven expression is higher than expression from any of the viral promoters. In *Drosophila* cells, HP-driven expression is activated more quickly and reaches higher levels of expression than observed in SF21 cells up to 24 h p.i. (compare Fig. 3A and 4D). Expression in Ld652Y cells is strikingly low for all promoters tested. (Note differences in the scales of the ordinates in all panels.) As might be expected in nonpermissive cells, VLP is the weakest among the viral promoters in driving expression. However, the LP rather than the EP drives the highest expression among the viral promoters tested in these cell lines.

Late and very late transcripts in nonpermissive cell lines. It was somewhat surprising to find evidence of LP and VLP

activity in nonpermissive cell lines. Late and very late baculovirus transcripts initiate from an essential and absolutely conserved TAAG sequence (35, 40, 46). Transcription from these TAAG sites is dependent on DNA replication, as determined by sensitivity to aphidicolin (42, 46), and is probably accomplished by a virus-induced α-amanitin-insensitive RNA polymerase activity (10, 21, 53). Genuine late and very late transcription in these nonpermissive cell lines would therefore indicate that the cells were able to support some level of viral DNA synthesis and that the virus was able to induce the α-amanitin-resistant RNA polymerase activity. However, it was possible that the CAT activity detected in the nonpermissive cell lines infected with vCapcat324 and vhcLSXIV was the result of aberrant transcription from sequences upstream of the LP and VLP.

To address this possibility, we proceeded to map the 5′ ends of the LP and VLP CAT transcripts to determine whether they are transcribed from genuine late and very late transcriptional start sites. The vp39 promoter includes three TAAG sites which are utilized in permissive SF21 cells (46). Primer extension analysis indicates that, except for the most-distal TAAG site in infected Dm cells, all three TAAG initiation sites are also utilized in the nonpermissive lines (Fig. 5A, arrows). Interestingly, the relative efficiency with which these three sites are utilized differs among the different cell lines. Very late transcription in these nonpermissive lines also initiates from the correct TAAG site (Fig. 5B, arrow).

We also wanted to determine whether late transcription in nonpermissive cells is subject to the same DNA synthesis requirement as in permissive cells. Each cell line was infected with vCapcat324 (LP) or with vETLcat (EP), and the cells were treated with the DNA synthesis inhibitor aphidicolin immediately after the viral adsorption period. Cells were harvested at 24 h p.i. and assayed for CAT activity. In permissive SF21 cells, aphidicolin prevents LP expression, keeping levels of CAT expressed from an LP below the level detectable in our standard biochemical CAT assay (Table 1). EP activity is not dependent on DNA synthesis, and therefore, CAT expression from the ETL promoter in vETLcat-infected cells was, as expected, relatively unaffected by aphidicolin treatment (Table 1). In all cell lines tested except Dm, aphidicolin treatment prevented the expression of detectable levels of CAT protein from an LP but not from an EP (Table 1).

Thus, in nonpermissive lepidopteran cell lines, the virus is able to achieve a significant amount of authentic late transcription which is dependent on DNA synthesis. It is unclear whether late expression in Dm cells is uncoupled from DNA synthesis or whether aphidicolin is ineffective in this cell line. A limited amount of Ac*M*NPV DNA replication does take place in infected Dm cells (2, 34a), and on the basis of primer extension analysis of late and very late CAT transcripts (Fig. 5), the viral LP and VLP are being utilized in infected Dm cells.

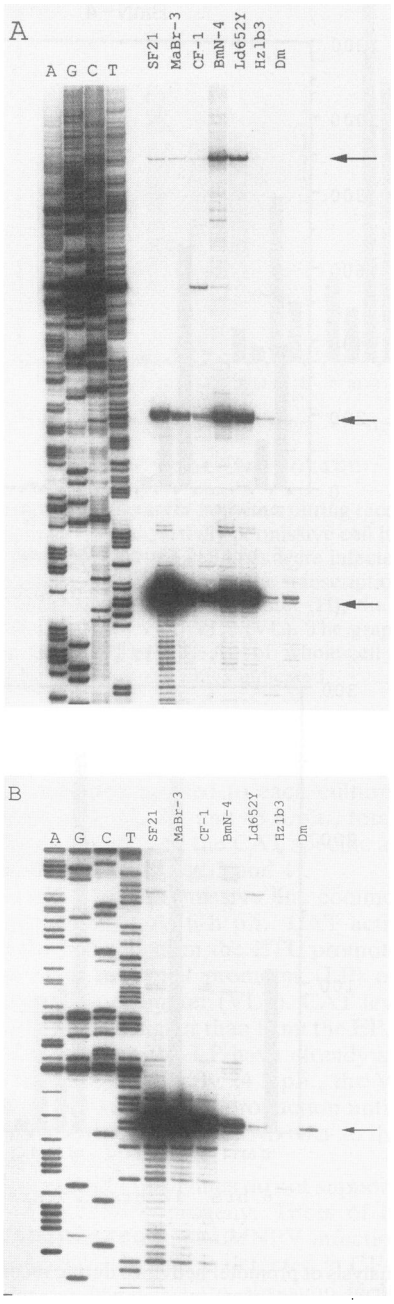


FIG. 5. Primer extension analysis of CAT mRNA transcribed from viral LPs and VLPs. Cell lines were infected with vCapcat324 (A) or vhcLSXIV (B) and harvested at 24 and 48 h p.i., respectively. Total-cell RNA was isolated and annealed to an end-labeled CAT-specific primer. The primer was extended by reverse transcription, and the products were analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography. The sequences presented (lanes A, G, C, and T) were generated with the same primer in a chain termination sequencing reaction with the appropriate plasmid. Arrows indicate the transcriptional start sites of CAT mRNAs.

DISCUSSION

We have investigated promoter-dependent expression of a virus-borne reporter gene in permissive and nonpermissive insect cells. We have confirmed and extended the observations of Carbonell et al. (2) that Ac*M*NPV is able to enter insect cells of a variety of taxa and that an important determinant of expression in a given cell line is the promoter used to drive expression. The modified polyhedrin VLP drives the highest level of CAT expression only in permissive cell lines. The promoters utilized by the host RNA polymerase II, namely, insect-derived promoters and viral EPs, provide much higher levels of gene product than VLPs in cell lines that do not support virus replication. The most remarkable example of this is CAT production in *Drosophila*

TABLE 1. Effects of aphidicolin treatment on viral EP and LP activity

Cell line	CAT activity[a] (% of untreated value)	
	EP	LP
SF21	87	<0.2
MaBr-3	14	<0.2
CF-1	18	<0.2
BmN-4	150	<0.2
Ld652Y	77	<0.2
Hz1b3	88	<0.2
Dm	78	26

[a] Values are the amount of CAT activity detected in aphidicolin-treated cells as a percentage of the CAT activity detected in untreated cells.

cells driven by the *Drosophila* HP; the levels of CAT produced in 24 h are nearly equivalent to those observed at 48 h in SF21 cells with the VLP.

Although some general trends in the data may prove to be predictive of promoter activity in other cell lines, the data demonstrate that each cell line behaves in a somewhat different manner, suggesting that the specific deficiencies which prevent productive infections are unique to each nonpermissive cell line. The most distinctive expression characteristics were observed in the Ld652Y cell line, which produced only low CAT activity with all the promoters tested (Fig. 4B; note different scales in each panel in Fig. 4). Our primer extension data, however, indicated that significant levels of late mRNAs were present in the Ld652Y cells. These data are consistent with prior reports that AcMNPV transcripts are poorly translated in this cell line early in infection (15) and that all protein synthesis, cellular and viral, is terminated by 20 h p.i. (15, 31). The nature of our experimental approach does not allow us to distinguish transcriptional from translational blocks in a quantitative manner. However, our data do reveal that the other nonpermissive insect cell lines tested are able to support significant levels of LP activity and, in most cases, some VLP activity. Thus, there is not an absolute block to late and very late transcription in the cell lines tested.

The LP and VLP activity we have detected in AcMNPV-infected *D. melanogaster* cells has not been reported previously. Carbonell et al. (2) did not observe VLP activity in β-galactocidase assays to detect reporter gene expression driven by the polyhedrin promoter. In addition, Northern (RNA blot) analysis (42) does not reveal specific late or very late transcripts in AcMNPV-infected *Drosophila* cells. However, we show that the use of more sensitive methods (radiometric CAT assays and primer extension analysis) reveals that both LPs and VLPs are functional in *Drosophila* cells (Fig. 4D and 5).

The ability to block expression from the LP with aphidicolin (Table 1) reveals that at least a limited amount of DNA replication occurs in the nonpermissive lepidopteran cell lines tested. Since aphidicolin blocks both host and viral DNA polymerases (34), it is not clear whether the replication that is occurring is directed by host enzymes or viral enzymes.

It has been suggested that baculovirus expression vectors which produce a foreign protein earlier in infection may be superior to expression vectors utilizing VLPs if the protein requires extensive uniform posttranslational modification (16, 46). The strongest and earliest expression of CAT was obtained with an insect promoter (*Drosophila* hsp70) rather than a viral EP (Fig. 3A). It may be that, in SF21 cells, a strong *S. frugiperda* promoter would produce even higher levels of foreign protein than the *Drosophila* promoter. However, by 12 h p.i., expression from a viral LP has already exceeded the expression observed with either the viral EP (by 7-fold) or the *Drosophila* promoter (by 2.5-fold) (Fig. 3A). Additionally, expression driven by the LP is only two- to threefold less than expression driven by the VLP. Therefore, in fully permissive cells, the vp39 promoter, alone or in combination with a VLP, may produce higher levels of more homogeneously modified protein by 24 to 36 h p.i. than could be produced from the polyhedrin promoter alone.

The relevance of our results to the design and safety of baculovirus pesticides will need to be explored in in vivo studies, but our present in vitro studies provide insight into some factors which should be considered in pesticide design and safety. Our data support the view of Carbonell et al. (2) that promoter choice may influence the range of insects affected by a recombinant virus expressing an insecticidal gene. VLPs are predicted to direct high-level expression of the gene only in permissive host insects, limiting the effectiveness of the recombinant viruses to their natural hosts. In contrast, the use of viral EPs and LPs as well as virus-borne insect promoters may allow expression of the gene in a wider range of insects than can be productively infected by the virus. However, expression of an insecticidal gene in cells contacted by infectious virus particles (i.e., cells at the primary site of infection, such as midgut epithelial cells) may not be sufficient to affect the health or behavior of nonpermissive insects. The level of effect will probably depend on the number of cells involved in the primary infection, the level of gene expression, the toxicity of the gene product, and the stability of the gene product in the insect.

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