In Vitro Transcription from Baculovirus Late Gene Promoters: Accurate mRNA Initiation by Nuclear Extracts Prepared from Infected *Spodoptera frugiperda* Cells†

BARBARA GLOCKER, RICHARD R. HOOPES, JR., LARRY HODGES, AND GEORGE F. ROHRMANN*

Department of Agricultural Chemistry, Agricultural and Life Sciences 1007,
Oregon State University, Corvallis, Oregon 97331-7301

Received 8 January 1993/Accepted 25 March 1993

Extracts prepared from nuclei of *Autographa californica* nuclear polyhedrosis virus-infected *Spodoptera frugiperda* cells were shown to support in vitro transcription from baculovirus late gene promoters. In vitro transcription was optimized for the late promoter of the 39K gene. The Mg^{2+} concentration was critical; concentrations higher than 1 to 2 mM did not support late transcription. Additional conditions included template (40 μ g/ml), extract (2.5 mg/ml), and incubation time (25 min). Using a combination of runoff assays and high-resolution primer extension analyses, this system was shown to accurately initiate transcription from a variety of baculovirus late gene promoters, including those from the 39K and p39/capsid late genes and the hyperexpressed p10 and polyhedrin very late genes. In vitro transcription from the 39K late promoter was resistant to high concentrations of both α -amanitin (100 μ g/ml) and tagetitoxin (4,000 U/ml), suggesting that neither RNA polymerase II nor III is responsible for the transcription of baculovirus late genes.

The *Baculoviridae* family of insect viruses is characterized by a complex replication cycle that culminates in the occlusion of virions in a crystalline protein matrix (1). The best-characterized baculovirus, the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus (AcMNPV), has a double-stranded, circular, supercoiled DNA genome of approximately 128 kbp. Progression through the AcMNPV infection cycle is governed by a cascade of early, late, and very late gene transcription (3). Baculovirus early transcription is mediated by the host cell RNA polymerase II (4, 8) and begins before the initiation of replication of the viral genome. In contrast, late transcription follows the initiation of viral DNA replication and is dependent on the presence of an RNA polymerase that (i) is α -amanitin resistant (4, 6, 9), (ii) has a unique subunit composition (14), (iii) recognizes a late promoter element ([A/G/T] TAAG) (1), and (iv) initiates transcription from within the promoter sequence. This polymerase very likely contributes to the remarkable level of transcription from the polyhedrin and p10 gene promoters that forms the basis for the use of baculoviruses as expression vectors.

Although the baculovirus expression system is widely used, little is known about the mechanism governing late gene transcription. The development of in vitro transcription systems that reflect in vivo gene transcription is crucial for the identification and analysis of factors involved in late gene transcription. We have recently reported that baculovirus early genes are accurately initiated by nuclear extracts from uninfected insect (*Spodoptera frugiperda* Sf9) and human (Namalwa) cells (14). Subsequently, we demonstrated that extracts prepared from baculovirus-infected cells 4 to 8 h postinfection (p.i.) activated the transcription of early genes (5). However, we found no evidence for transcription from late promoter elements with extracts produced from cells

late in infection. In addition, a DNase activity that interferes with the analysis of in vitro transcription was detected in nuclear extracts produced from cells after 12 h p.i. (5).

In this report, we describe conditions for in vitro transcription from late baculovirus gene promoters, using nuclear extracts from AcMNPV-infected Sf9 cells.

MATERIALS AND METHODS

Templates used for in vitro transcription. (i) **39K gene promoter (pAc39K).** Plasmid pAc39K (Fig. 1B) contains a 1-kb *Pst*I-*Sst*I fragment derived from the AcMNPV *Pst*I K fragment (7) cloned into Bluescript (pKS⁻; Stratagene Cloning Systems) and was previously called p39K (5). The 39K gene has both early and late promoters (Fig. 1B).

(ii) **p39/capsid gene promoter (pAcp39).** A 2-kb *Bal*I fragment containing the AcMNPV p39/capsid gene was isolated and cloned into *Sma*I-digested pKS⁻. To create pAcp39 (Fig. 4B), a *Bam*HI-*Nar*I fragment was removed and ligated into *Bam*HI-*Cla*I-digested pKS⁻.

(iii) **p10 gene promoter (pAcp10).** Plasmid pAcp10 contains the AcMNPV p10 promoter region (Fig. 4D). This plasmid was constructed by cloning an *Eco*RV-*Bgl*II (nucleotides [nt] -308 to 951 [10]) fragment into pKS⁻ digested with the same two enzymes.

(iv) **Polyhedrin gene promoter (pAcPH).** To construct pAcPH, a 1-kb *Sal*I-*Bam*HI fragment containing the promoter region of the AcMNPV polyhedrin gene was cloned into pKS⁻ (Fig. 4F).

Cells, viruses, and infection of cells. *S. frugiperda* Sf9 cells (ATCC CRL 1711), grown in serum-free SF900II medium (GIBCO-Bethesda Research Laboratories) to a density of 2×10^6 cells per ml, were infected with AcMNPV (E-2 strain) at a multiplicity of infection of 10 (5). To initiate infection, Sf9 cells were pelleted and resuspended in 1/10 volume of the original medium, and virus was added, defining the 0-h time point. After rocking for 1 h at room temperature, the remainder of the original medium was added, and the culture

* Corresponding author. Electronic mail address: rohrmann@bcc.orst.edu

† Technical report no. 10,067 from the Oregon State University Agricultural Experiment Station.

FIG. 1. Analysis of in vitro transcription from AcMNPV 39K gene promoters. (A) Runoff assays of RNA produced by using a nuclear extract prepared at about 12 h p.i. Lanes: 1 and 2, *PvuII*-digested pAc39K with and without α -amanitin, respectively; 3 and 4, *SstI*-digested pAc39K with and without α -amanitin, respectively. The solid and dashed arrows on the right indicate the positions and sizes of the late and early transcription products, respectively; numbers on the left indicate positions of selected radiolabeled *HaeIII*-digested ϕ X174 markers. (B) Diagram of the pAc39K template. The arrows indicate the transcripts produced from the early (TATA) and late (ATAAG) promoters from *SstI*- and *PvuII*-digested templates, and the numbers indicate the predicted sizes of runoff transcripts; the cross-hatched bars indicate vector DNA. (C) Primer extension analysis of in vitro RNA transcribed from the pAc39K template in the presence of α -amanitin. The primer extension products were run on a sequencing gel alongside a DNA sequencing ladder of pAc39K generated with the same primer. Primer extension from RNA transcribed from *PvuII*-digested (lane 1) and undigested (lane 2) p39K templates is shown.

was incubated on an orbital shaker at 27°C (8) until the appropriate time postinfection.

Nuclear extract preparation. Nuclear extracts were prepared from AcMNPV-infected Sf9 cells at the times indicated, using a previously described method (8) modified by the addition of two ammonium sulfate precipitation steps. Sf9 cells were harvested by pelleting at 1,500 rpm for 10 min, resuspended in 4 packed-cell volumes of buffer A (10 mM Tris-HCl [pH 7.9], 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol), and left on ice for 10 min. The swollen cells were lysed by 8 strokes of a Kontes Dounce homogenizer (B pestle) and subjected to low-speed centrifugation (1,500 rpm, 10 min). The nuclei were resuspended in 1 nuclear pellet volume of a 9:1 mixture of buffer C (20 mM Tris-HCl [pH 7.9], 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol) and saturated (NH₄)₂SO₄ solution. Nuclei were treated with 12 strokes of a Kontes Dounce homogenizer (B pestle), transferred to ultracentrifuge tubes, and rocked on ice for 30 min. The suspension was centrifuged in a Beckman 100.2 rotor at 100,000 $\times g$ for 1 h at 4°C. The supernatant was removed, and saturated (NH₄)₂SO₄ was added in small aliquots to give a final concentration of 55%. After 30 min of incubation (with rocking) on ice, the precipitate was collected (15,000 $\times g$ for 30 min at 4°C), and the pellet was dissolved immediately in dialysis buffer (20 mM Tris-HCl, 100 mM KCl, 20% glycerol, 0.2 mM EDTA, 10 mM 2-mercaptoethanol). After dialysis for 3 h with two buffer changes, the extract was cleared by

microcentrifugation for 2 min. The supernatant was aliquoted, frozen in liquid nitrogen, and stored at -80°C. Extracts prepared in this manner are stable for at least 6 weeks.

Runoff transcription assays, primer extension analysis, and DNA sequencing. Standard runoff assays in 20 μ l (final volume) were done with DNA template and nuclear extract concentrations of 37.5 μ g/ml and 2.5 mg/ml, respectively, in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 8.4 at 25°C) as described previously (8). RNasin (1 U/ μ l, final concentration; Promega, Inc.) was included in all transcription reactions. Early conditions refer to preincubation for 15 min at 30°C prior to the addition of the nucleotides with a buffer containing 4 to 6 mM MgCl₂. Under late conditions, mixtures containing the buffer, DNA template, and nuclear extract were incubated on ice for 15 min, and the final MgCl₂ concentration was 1 to 2 mM. Transcription was initiated by the addition of nucleotides (600 μ M each ATP, CTP, and GTP; 25 μ M UTP (Pharmacia); 5 μ Ci of [α -³²P]UTP [DuPont/NEN]) and incubated for 25 min at 30°C. The specific transcription conditions are indicated in the figure legends. When tested, the transcription inhibitors α -amanitin (Sigma) and tagetitoxin (Epicentre Technologies) were added to the reactions before the nucleotides.

Primer extension analysis of in vitro-generated RNA was carried out as previously described (5). The following primers, complementary to sequences of the vector pKS⁺, were

used: for 39K, 3'-CGCCAGGGTTTTCCAGTCACGAC-5' (forward primer; Promega); for p10 and polyhedrin, 3'-CTAGGTGATCAAGATCT-5' (SK primer; Stratagene); and for p39/capsid, 3'-TCACACAGGAAACAGCTATGAC-5' (reverse primer, Promega, Inc.). Primer extension products were resolved on sequencing gels next to a sequencing ladder generated by using the corresponding template and primer. DNA sequencing was performed with the Sequenase sequencing kit (United States Biochemical Corp.) and [α - 35 S]ATP (NEN, Inc.) according to the manufacturers' instructions.

RESULTS

In vitro transcription from the late baculovirus 39K promoter. Plasmid pAc39K, which contains the promoter region of the 39K gene, was used to determine the conditions for in vitro transcription from a baculovirus late promoter. The 39K gene has both early and late promoter elements that are separated by 25 nt (Fig. 1B). The presence of promoters transcribed by different polymerases allowed for comparison of both early and late transcription conditions for the same template. Although a variety of conditions were tested, we identified two parameters for late gene transcription that differed from conditions previously shown to be optimal for RNA polymerase II transcription (8): altering the preincubation step and reducing the Mg^{2+} concentration. Both of these changes may minimize the effects of the DNase present in nuclear extracts prepared late in infection. The infected-cell nuclear extracts were also subjected to two ammonium sulfate precipitations in an attempt to eliminate the DNase. Although these steps reduced the total protein concentration by enriching for proteins of intermediate size, the DNase activity remained (data not shown). Since these extracts were active for late transcription, the ammonium sulfate precipitation steps were incorporated into the protocol in anticipation of fractionation of the extract.

Transcription from the 39K late promoter by nuclear extracts prepared at about 12 h p.i. is shown in Fig. 1A. In vitro transcription in the presence of α -amanitin with use of *Pvu*II- and *Sst*I-digested pAc39K templates produced runoff transcripts of about 364 and 239 nt, respectively (Fig. 1A, lanes 1 and 3). These sizes correspond to those expected for transcription from the late promoter element (Fig. 1B). In the absence of α -amanitin, early transcripts of about 339 and 214 nt, respectively, were also produced from these templates (Fig. 1A, lanes 2 and 4). Late transcription was unaffected by the presence (lanes 1 and 3) or absence (lanes 2 and 4) of α -amanitin, an inhibitor of RNA polymerase II. A variety of nonspecific bands are present in the transcription reactions. These bands were shown to be sensitive to RNase (5) and may originate from the labeling of endogenous RNA species by an enzyme present in the nuclear extracts.

To confirm that the runoff signal corresponded to initiation from the late promoter element, primer extension analysis was done on RNA transcribed from the pAc39K template, using late in vitro transcription conditions in the presence of α -amanitin. A primer complementary to plasmid sequences was used to ensure that primer extension was specific to in vitro-generated 39K RNA and would not prime 39K RNA which may be present in the infected-cell nuclear extracts. The RNA was found to initiate at the first A of the late promoter element ATAAG, confirming that late in vitro-generated transcripts were initiating at positions similar to those produced in vivo (Fig. 1C). No difference in levels of

FIG. 2. Comparison of in vitro transcription conditions for the 39K early and late promoters in a runoff assay using early (15-min preincubation at 30°C, 6 mM $MgCl_2$; lanes 1 to 4) and late (no preincubation at 30°C, 2 mM $MgCl_2$; lanes 5 to 8) transcription conditions. Shown are transcription by uninfected-cell nuclear extracts in the presence (lanes 1 and 5) or absence (lanes 2 and 6) of α -amanitin and transcription by infected-cell nuclear extracts (prepared at about 12 h p.i.) in the presence (lanes 3 and 7) or absence (lanes 4 and 8) of α -amanitin. The solid and dashed arrows indicate the positions and sizes of the late and early runoff transcripts, respectively (see Fig. 1B). Markers are the same as for Fig. 1A.

primer extension products was observed with use of an undigested (lane 1) or linear (lane 2) template.

Comparison of early and late in vitro transcription. We compared the capacity of nuclear extracts prepared from uninfected and infected cells to support transcription from the early and late promoters of an *Sst*I-digested 39K template. Both early (preincubation at 30°C for 15 min; 4 to 6 mM $MgCl_2$) and late (preincubation on ice for 15 min; 1 to 2 mM $MgCl_2$) conditions (Fig. 2) were tested. Under early conditions with a nuclear extract prepared from uninfected Sf9 cells, we detected a strong transcription signal from the 39K promoter (Fig. 2, lane 2) which was sensitive to α -amanitin (lane 1). When transcription by an extract from 12 h p.i. was tested under early conditions, only a weak α -amanitin-sensitive early transcription signal (lanes 3 and 4) was obtained. No late transcripts were detected. When late transcription conditions were used with uninfected-cell extracts, an early α -amanitin-sensitive transcript (lanes 5 and 6) was produced but no late transcription was observed. However, late conditions were suboptimal for early transcription (compare lane 6 with lane 2). When late transcription conditions were used with nuclear extracts prepared 12 h p.i., both early (214-nt) and late (239-nt) transcripts were present (lane 8). As expected, the late transcript is insensitive to α -amanitin (compare lanes 7 and 8).

Optimization of conditions for late in vitro transcription. Once we identified conditions that supported late transcription, we optimized several reaction parameters (Fig. 3). For these investigations, a nuclear extract prepared at about 12 h p.i. and *Sst*I-digested pAc39K template DNA were used (Fig. 1B).

The most striking difference between early and late transcription was obtained by varying the Mg^{2+} concentration (Fig. 3A). Late transcription is supported best by low magnesium concentrations (1 or 2 mM Mg^{2+} ; Fig. 3A, lanes 1 and 2). Higher concentrations (4 and 6 mM) that are in a range optimal for early RNA polymerase II-mediated transcription (lanes 4 and 6) did not support late transcription.

FIG. 3. Optimization of late in vitro transcription conditions, using an infected-cell nuclear extract. (A) MgCl₂ concentration. Lane 6, transcription with 2 mM MgCl₂ in the presence of 2 μg of α-amanitin per ml. (B) Template concentration. (C) Nuclear extract concentration. (D) Incubation time. For all reactions, the extract was prepared at about 12 h p.i. and an *Sst*I-digested pAc39K template was used (see Fig. 1B). For details, see the legend to Fig. 2. Standard late transcription conditions were used and varied as indicated.

The template concentration for transcription from the late promoter was near optimal over a wide range from 30 to at least 50 μg/ml (Fig. 3B, lanes 3 to 6). At the higher template concentrations (50 μg/ml) (lanes 2 to 6), the intensity of background bands increased. The optimal template concentrations were similar for both early and late transcripts (Fig. 3B).

Nuclear extract concentrations of 1.2 to 3.7 and 2.5 to 3.7 mg/ml gave optimal levels of transcription from late and early promoters, respectively (Fig. 3C). Incubations for 25 to 50 min were in the optimal range for transcription from both promoters (Fig. 3D).

In vitro transcription from the p39/capsid, p10, and polyhedrin promoters. To determine whether the conditions that we developed for in vitro transcription from the 39K gene late promoter were applicable to transcription from other baculovirus late promoters, we examined in vitro transcription from the late p39/capsid and from the very late p10 and polyhedrin promoters.

The p39/capsid gene contains three late promoters (Fig. 4B), and RNAs initiate at all three sites in vivo (13). This template showed higher background in runoff assays than did the 39K gene and was therefore analyzed by primer extension using RNA transcribed in vitro by a nuclear extract prepared at about 16 h p.i. Transcription initiation was observed from the two promoters most proximal to the p39/capsid open reading frame (Fig. 4A). Although in vitro transcripts did not map to the most distal promoter (data not shown), in vivo transcription occurs from this promoter at a reduced level (13), suggesting that it is suboptimal in its position or sequence context and therefore is not detected by the in vitro system.

The p10 gene is expressed in vivo at high levels very late in infection. Runoff assays in the presence of α-amanitin show signals of about 360 and 235 nt, the expected sizes for late promoter runoff transcripts from *Pvu*II- and *Xba*I-digested templates (data not shown). Primer extension analysis confirmed that in vitro-generated p10 RNA was initiating

FIG. 4. In vitro transcription from late promoters of p10, p39/capsid, and polyhedrin genes. (A) Primer extension analysis of in vitro-transcribed p39/capsid RNA. A 16-h-p.i. nuclear extract was used. Primer extension from RNA transcribed from *Pvu*II-digested (lane 1) and nonlinearized (lane 2) templates is shown. (B) Diagram of the p39/capsid gene promoter region. (C) Primer extension analysis of in vitro-transcribed p10 RNA. A 24-h-p.i. nuclear extract and nonlinearized pAcP10 template were used. (D) Diagram of the p10 gene promoter region. (E) Primer extension analysis of in vitro-transcribed polyhedrin RNA. A 20-h-p.i. nuclear extract and nonlinearized pAcPH template were used. (F) Diagram of the polyhedrin gene promoter region. Template diagrams are not to scale. Hatched bars indicate vector DNA. All in vitro RNA for primer extension analysis in this figure was generated in the presence of α -amanitin (2 μ g/ml).

at the A of the p10 ATAAG late promoter sequence (Fig. 4C).

The polyhedrin promoter, used as the basis of most baculovirus expression vectors, contains the late promoter motif ATAAG (Fig. 4F) but is transcribed later than most other late genes and at much higher levels. To determine whether our late transcription system also supports transcription from the polyhedrin gene promoter, we analyzed RNA transcribed from pAcPH template DNA by a nuclear

extract prepared at about 20 h p.i. Although runoff transcripts could not be detected, primer extension analysis indicated that specific transcription was initiating from the first A of the late promoter motif ATAAG (Fig. 4E). The reason for the low level of polyhedrin transcription is not clear. However, our nuclear extraction protocol and transcription conditions were optimized for the 39K promoter, and these conditions may not be optimal for polyhedrin transcription.

Baculovirus late transcription is not mediated by RNA polymerase II or III. It has been previously demonstrated that the late baculovirus polymerase is α -amanitin resistant (6). We found that in vitro transcription from late promoters was also not affected by high concentrations of α -amanitin (100 μ g/ml) (Fig. 5, lane 3); however, low concentrations of α -amanitin (2 μ g/ml) inhibited early transcription of the 39K gene (lane 2).

Recently it was reported that the promoter region of p10 and polyhedrin genes from a number of baculoviruses have sequence similarity to genes transcribed by RNA polymerase III (15). Although RNA polymerase III from insects is not sensitive to high concentrations of α -amanitin, tagetitoxin was shown to specifically block elongation of transcription by RNA polymerase III from extracts of *Bombyx mori*, HeLa, and *Saccharomyces cerevisiae* cells (12). To determine whether RNA polymerase III is involved in baculovirus late transcription, increasing concentrations of tagetitoxin were included in a series of late transcription reactions. We found no evidence for inhibition of late transcription by tagetitoxin in the concentrations reported to interfere with RNA polymerase III transcription (2,000 U/ml) from other systems. Although high concentrations (4,000 and 8,000 U/ml) of tagetitoxin caused reduced levels of RNA polymerase II transcription (Fig. 5, lanes 6 and 7), late transcription was unaffected (lanes 6 and 7).

FIG. 5. Effects of α -amanitin and tagetitoxin on baculovirus transcription. Lanes: 1, control, no inhibitor included; 2 to 7; α -amanitin and tagetitoxin concentrations as indicated. Standard in vitro transcription reactions (20 μ l) employing late conditions with *Ssr*I-digested pAc39K template, 1 mM $MgCl_2$, and a nuclear extract prepared at about 12 h p.i. were used. For details, see the legend to Fig. 2.

DISCUSSION

To develop a system capable of supporting *in vitro* transcription of baculovirus late genes, we focused on transcription from a DNA template containing the 39K gene, which has early and late promoters. This strategy allowed for the comparison of conditions optimal for both early and late transcription. We found that elimination of 30°C preincubation of the template with nuclear extracts was essential for detecting late transcription. This change may minimize exposure of the DNA template to the DNase present in infected-cell extracts. The baculovirus late polymerase showed a Mg^{2+} optimum of 1 to 2 mM under the conditions that we used. In contrast, other polymerases, such as RNA polymerase II (2) and vaccinia virus RNA polymerase (11), show optimal Mg^{2+} concentrations of 5 to 15 and 2 to 8 mM, respectively. We found that these transcription conditions were also capable of supporting accurate initiation of transcription from the promoters of another late gene (p39/capsid) and two very late hyperexpressed genes (p10 and polyhedrin).

We demonstrated that *in vitro* late transcription is insensitive to α -amanitin (100 μ g/ml) and therefore is not likely to be dependent upon the host RNA polymerase II. These results are in agreement with those originally reported by Grula et al. (6). We also found by examining transcription from a late promoter in the presence of tagetitoxin that concentrations much higher (>2,000 U/ml) than those inhibitory for RNA polymerase III from extracts of HeLa, *S. cerevisiae*, and *B. mori* cells (12) had no effect on late baculovirus transcription. Because of the lack of apparent species differences in RNA polymerase III sensitivity, these data suggest that RNA polymerase III is not involved in baculovirus late transcription.

The development of an *in vitro* transcription system for baculovirus late genes will be useful for the analysis of baculovirus transcription. It will provide a means for assaying the components of fractionated nuclear extracts. Once active components are identified, further purification of these fractions should lead to the identification of the components responsible for baculovirus late transcription. This approach will likely become an essential tool for defining the mechanism by which baculoviruses regulate late gene transcription.

ACKNOWLEDGMENTS

We thank C. Rasmussen, J. Funk, and D. Leisy for suggestions and criticisms of the manuscript.

This work was supported by grant AI 21973 from the NIH.

REFERENCES

1. Blissard, G. W., and G. F. Rohrmann. 1990. Baculovirus diversity and molecular biology. *Annu. Rev. Entomol.* **35**:127–155.
2. Dignam, J. D., R. M. Lebowitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**:1475–1489.
3. Friesen, P. D., and L. K. Miller. 1986. The regulation of baculovirus gene expression. *Curr. Top. Microbiol. Immunol.* **131**:31–49.
4. Fuchs, Y. L., 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.* **48**:641–646.
5. Glocker, B., R. R. Hoopes, and G. F. Rohrmann. 1992. *In vitro* transactivation of baculovirus early genes by nuclear extracts from *Autographa californica* nuclear polyhedrosis virus-infected *Spodoptera frugiperda* cells. *J. Virol.* **66**:3476–3484.
6. Grula, M. A., P. L. Buller, and R. F. Weaver. 1981. Alpha amanitin-resistant viral RNA synthesis in nuclei isolated from nuclear polyhedrosis virus-infected *Heliothis zea* larvae and *Spodoptera frugiperda* cells. *J. Virol.* **38**:916–921.
7. Guarino, L. A., and M. W. Smith. 1990. Nucleotide sequence and characterization of the 39K gene region of *Autographa californica* nuclear polyhedrosis virus. *Virology* **179**:1–8.
8. Hoopes, R. R., Jr., and G. F. Rohrmann. 1991. *In vitro* transcription of baculovirus immediate early genes: accurate mRNA initiation by nuclear extracts from both insect and human cells. *Proc. Natl. Acad. Sci. USA* **88**:4513–4517.
9. 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.
10. Liu, A., J. Qin, C. Rankin, and R. R. Weaver. 1986. Nucleotide sequence of a portion of the *Autographa californica* nuclear polyhedrosis virus genome containing the EcoRI site-rich region (hr5) and an open reading frame just 5' of the p10 gene. *J. Gen. Virol.* **67**:2565–2570.
11. Rohrmann, G., and B. Moss. 1985. Transcription of vaccinia virus early genes by a template dependent soluble extract of purified virions. *J. Virol.* **56**:349–355.
12. Steinberg, T. H., D. E. Mathews, R. D. Durbin, and R. R. Burgess. 1990. Tagetitoxin: a new inhibitor of eukaryotic transcription by RNA polymerase III. *J. Biol. Chem.* **265**:499–505.
13. Thiem, S. M., and L. K. Miller. 1989. Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **63**:2008–2018.
14. Yang, C. L., D. A. Stetler, and R. F. Weaver. 1991. Structural comparison of the *Autographa californica* nuclear polyhedrosis virus-induced RNA polymerase and the three nuclear RNA polymerases from the host, *Spodoptera frugiperda*. *Virus Res.* **20**:251–264.
15. Zanotto, P. M. D. A., M. J. A. Sampaio, D. W. Johnson, T. L. Rocha, and J. E. Maruniak. 1992. The *Anticarsia gemmatilis* nuclear polyhedrosis virus polyhedrin gene region: sequence analysis, gene product and structural comparisons. *J. Gen. Virol.* **73**:1049–1056.