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# *In Vitro* Transcription Analyses of *Autographa californica* Nuclear Polyhedrosis Virus Genes

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Cell-free extracts prepared from cultured insect cells, *Spodoptera frugiperda*, were analyzed for activation of early gene transcription of an insect baculovirus, *Autographa californica* nuclear polyhedrosis virus (AcNPV). The template DNA used for *in vitro* transcription assays contained promoter sites for the baculovirus genes that have been classified as immediate early (α) or early genes. These genes are located in the *Hin*dIII-K/Q region of the AcNPV genome. Nuclei isolated from the AcNPV-infected *Spodoptera frugiperda* cells were also used for *in vitro* transcription analysis by RNase-mapping the labeled RNA synthesized from *in vitro* run-on reaction in the isolated nuclei. The genes studied by this technique were p26 and p10 genes which were classified as delayed early and late gene, respectively. We found that transcription of the genes from the *Hin*dIII-K region was accurately initiated and unique in the whole cell extract obtained from uninfected cells, although abundance of the *in vitro* transcripts was reverse to that of *in vivo* RNA. With isolated nuclei transcription of the p26 gene was inhibited by α-amanitin suggesting that the p26 gene was transcribed by host RNA polymerase II. However, transcription of the p10 gene in isolated nuclei was not inhibited by α-amanitin, but rather stimulated by the inhibitor. We also found that the synthesis of α-amanitin-resistant RNA polymerase was begun before 6 hr p.i., the time point at which the onset of viral DNA replication as well as the appearance of α-amanitin-resistant viral transcripts were detected. These studies give us strong evidence to support the previous data that early genes of AcNPV were transcribed by host RNA polymerease III, while transcription of late genes was mediated at least by a novel α-amanitin-resistant RNA polymerase.

The baculovirus *Autographa californica* polyhedrosis virus (AcNPV), a genus of the family of Baculoviridae, is a rod-shaped DNA-containing virus that infects lepidopteran insects and replicates in their nuclei in nature. The genome of AcNPV is a double-stranded, circular, supercoiled DNA of about 128 kb. Viral DNA replication begins by 6 hr post-infection (p.i.) and eventually culminates in the occlusion of virions in a crystalline protein matrix (1, 18). Progression through the AcNPV infection cycle is governed by a cascade of viral gene expression (5).

The expression of the AcNPV genes is temporally regulated (12, 19) and the protein synthesis has been divided into four main phases according to appearance of proteins as follows: immediate early (α) appearing 2 hr p.i., delayed early (β) appearing 6 hr p.i., late (ρ) appearing 12 hr p.i., and very late (δ) appearing 24 hr p.i. (12). Immediate early (α) genes are expressed without prior synthesis of viral proteins. Expression of delayed early genes requires the synthesis of at least one immediate early gene product. Late gene expression not only requires synthesis of and/or β proteins but also is dependent upon viral DNA replication (11, 18). The δ genes are associated with viral occulusion and are highly expressed after release of extracellular virus. Transcripts may also be classified this way, but they do not fall neatly into four phases. Instead, we can identify early transcripts as those which appear before 6 hr p.i. (the onset of viral DNA replication) and late transcripts as those which appear after this time (10).

Transcription of early genes is mediated by the host cell RNA polymerase II (7, 9, 10), and begins before the initiation of replication of the viral genome. In contrast, late transcription follows the initiation of viral DNA replication, simultaneous with the beginning of the late phase of infection. The onset of late gene transcription, however, is dependent upon the presence of a novel RNA polymerase that (i) is α-amanitin resistant (7, 8, 10), (ii) has a unique subunit composition (20), and (iii) recognizes a late promoter element. This RNA polymerase are estimated to contribute to the remarkable level of

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transcription from polyhedrin and p10 promoters that forms the basis for the use of baculoviruses as expression vectors (1). However, it is not clear yet whether this virus-induced polymerase is a virus-encoded enzyme, a virus-modified host enzyme, or even a virus-induced host enzyme (10).

Although the baculovirus expression system is widely used, little is known about the mechanism governing viral 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 viral gene transcription. Our earlier studies were carried out by *in vitro* run-on reactions for preparing labeled nuclear RNA in isolated nuclei followed by Southern blot analyses of these labeled RNAs using as probes. The works revealed that the onset of α-amanitin-resistant transcription was just after 6 hr p.i., coincident with the initiation of viral DNA replication in *Spodoptera frugiperda* cells infected with AcNPV. They also showed that transcripts from the p26 gene in the *Hin*dIII Q/P region and transcripts in the *Hin*dIII K/Q region are synthesized by the host RNA polymerase II early in infection. On the other hand, transcripts of the p10 gene in the *Hin*dII Q/P region are synthesized by the α-amanitin-resistant, virus-induced RNA polymerase late in infection (10, 11). In order to verify those observations further, we carried out another works employing more accurate methods as those of RNase mapping analyses of nuclear RNA labeled by *in vitro* run-on reactions with isolated nuclei in the presence or absence of α-amanitin, or of *in vitro* transcription assays using cell-free extracts such as whole cell extracts or nuclear extracts. Hereby, in this report we describe the results obtained from the works we have done on the *in vitro* transcription analyses as mentioned.

## MATERIALS AND METHODS

### Cells and Virus

*Spodoptera frugiperda* (IPLB-SF-21) cells were cultured in TC-100 medium supplemented with 2.5 mg of tryptose broth per ml and 10% fetal bovine serum. Cell monolayers ($2\times10^7$ cells per 100-mm plate) were inoculated with non-occluded virions of the L1 strain of AcNPV at a multiplicity of 20 p.f.u. per cell. The virus used for infection of *S. frugiperda* cells was from the first passage of the L1 variant. After 1 hour adsorption at room temperature, the residual inoculum was removed and the monolayers were washed twice with TC-100. Fresh growth medium (6 m*l*) was added, and incubation was continued at 27°C (4). Zero time postinfection (p.i.) was defined as the point at which fresh growth medium was added to cells.

### Purification of RNA Polymerases from *S. Frugiperda* Cells.

RNA polymerases were purified from the uninfected or infected *S. frugiperda* cells with AcNPV by the procedure of Fuchs *et al.* (7) with three ion exchange columns (Phospho-Cellulose, DEAE-Cellulose, and DEAE-Sephadex columns) consecutively. Activity was measured as described previously (7).

### *In vitro* Transcription Analysis

A) Preparation of whole cell extracts: Suspension cultures of *S. frugiperda* cells were grown to a density of $3\times10^6$ cells per m*l* at 27°C. Whole cell extracts were prepared by the method of Manley *et al.* (14) with a slight modification of their procedure in which the ammonium sulfate concentration used in preparation of the extracts was 45% saturated. *In vitro* transcription reactions with the whole cell extracts were performed under standard conditions as previously described (14).

B) Preparation of nuclear extracts: Nuclear extracts were prepared according to the procedure of Dignam *et al.* (3). *In vitro* run-off assays with the nuclear extract were performed by the procedure of Dignam *et al.* (3) with the exception that 12.5 μ*l* of the extract were assayed in a total volume of 25 μ*l*. The final concentrations of the ribonucleoside triphosphates in the reactions were as followings: 0.6 m*M* each of the three unlabeled ATP, CTP, and UTP, 60 μM of GTP including 20 μCi of α-$^{32}$P-GTP, respectively. For each preparation of extract, the optimal density of template DNA was determined in order to obtain specific and efficient recognition of promoter sequences. Transcription was allowed to proceed for 60 minutes at 30°C. Following the incubation, the reactions were terminated, and the RNA was extracted and analyzed by PAGE on gels containing 7 M urea (10). Autoradiography of the gel was carried out by the standard protocol.

C) Nuclear run-on assay: Isolation of nuclei from *S. frugiperda* cells and nuclear run-on assay with the isolated nuclei in the presence of α-$^{32}$P-GTP were performed as described (10). *In vitro* labeled nuclear transcripts obtained by allowing pre-initiated RNA chains to elongate *in vitro* by the method of Osborne *et al.* (15) with some modifications previously described (10) were chromatographed on a Sephadex G-75 column to remove unincorporated α-$^{32}$P-GTP. The fractions containing labeled RNA were pooled, and mapped with antisense RNA probes by the procedure of Peterlin *et al.* (16) with exceptions described in the text.

## RESULTS AND DISCUSSION

### Isolation of a Novel RNA Polymerase

Fuchs *et al.* (7) reported that up to 8 hr postinfection

(p.i.) most of the viral RNA synthesis remained sensitive to 5 μg of α-amanitin per ml in *Spodoptera prugiperda* cells infected by *Autographa californica* nuclear polyhedosis virus (AcNPV). During the course of infection, however, this sensitivity decreased, and at 24 hr p.i. RNA synthesis was completely resistant to the same concentration of α-amanitin. DEAE-Sephadex profiles of RNA polymerase isolated at 24 hr p.i. showed the presence of a new, chromatographically distinct, α-amanitin-resistant form. However, they did not investigate by the DEAE-Sephadex column chromatography how early the novel RNA polymerase was started synthesizing, or whether at 8 hr p.i. the novel RNA polymerase was already sythesized in the cells infected by AcNPV.

Progression through the AcMNPV infection cycle is governed by a cascade of ealy and late gene transcription (5). Baculovirus early transcription is defined as those which begins before the onset of replication of the viral genome, and is mediated by the host RNA polymerase II (7, 9, 10). In contrast, the late transcription follows the onset of viral DNA replication, and is mediated by a novel RNA polymerase that is α-amanitin resistant (7, 8, 10). In cell culture, viral DNA synthesis begins about 6 hr p.i. and proceeds for at least another 12 hr (2). Therefore,in order to determine whether the novel, virus infected-cell-specific RNA polymerase was synthesized even during the early phase of infection, cells infected for 6 hr were used to isolate RNA polymerases.

DEAE-Sephadex column chromatography was performed to isolate RNA polymerases from uninfected cells. (Fig. 2, panel A), or from the cells infected for 6 hr (panel B) and 24 hr (panel C), respectively. Three chromagraphycally distinct enzymes were isolated from uninfected cells; these were believed to be RNA polymerases I, II, III, respectively, which were consistent with those of the typical patterns revealed by Fuchs *et al.* (7). However, at as early as 6 hr p.i. an additional peak of RNA polymerase, which did not appear in the uninfected cells, was detected as a small amount of α-amanitin-resistant activity compared to the RNA polymerase II in the third peak which was sensitive to 2 μg per ml of α-amanitin (Fig. 2, panel B). At 24 hr p.i., the amount of this α-amanitin-resistant RNA polymerase was increased to as great as total of RNA ploymerase II

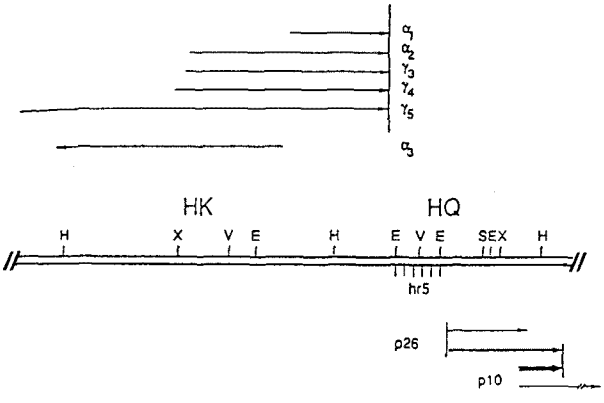


**Fig. 1.** Organization of overlapping early (α) and late (γ) transcripts mapping to the *Hin*dIII-K/Q regions (6, 17).
The transcripts are deginated by single-headed arrows above and below the map. p26 and p10 indicate mRNA encoding 26 and 10 kilodalton polypeptides, respectively. Their locations and relative abundance are described by the arrows. HK and HQ are the *Hin*dIII-K and *Hin*dIII-Q fragments of the AcNPV genome, respectively. Restriction sites: H, *Hin*dIII; X, *Xho*I; V, *Eco*RV; E, *Eco*RI; S, *Sal*I.

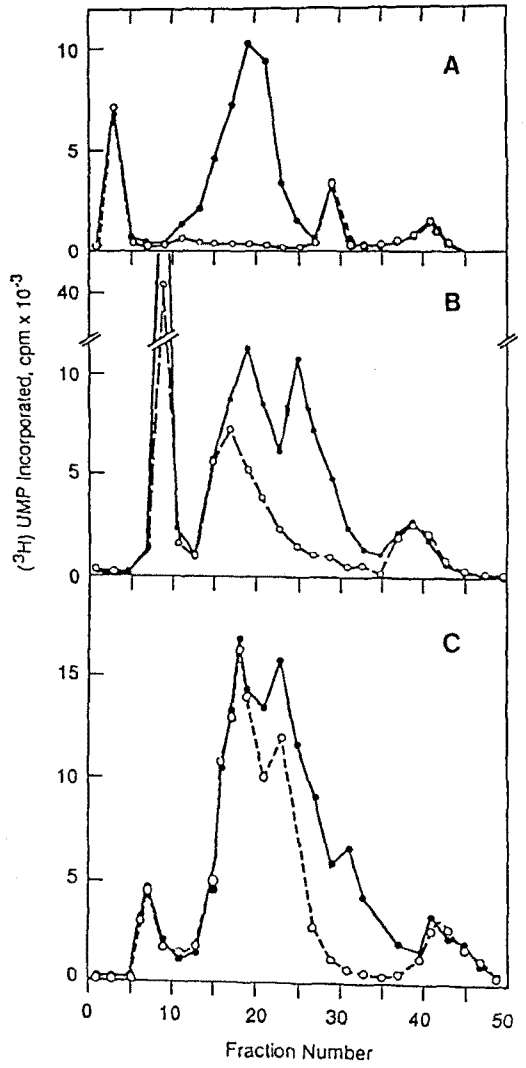


**Fig. 2.** DEAE-Sephadex elution profile of RNA polymerases.
RNA polymerases were purified from the uninfected *Spodoptera frugiperda* cells (panel A), or from the cells infected for 6 hr (panel B) and for 24 hr (panel C) with AcNPV. Purification was performed by the standard procedure using three ion exchange columns (phosphocellulose, DEAE-celluse, and then DEAE-Sephadex) consecutively as described (7). RNA polymerase activity in column fractions were assayed in the absence (●), and presence (○) of 2 μg/ml α-amanitin.

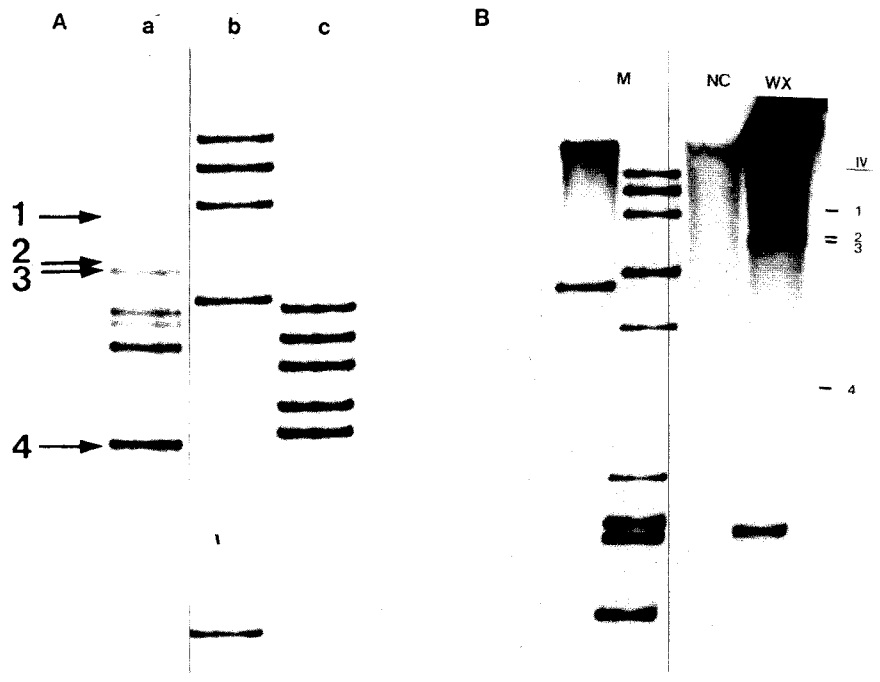


**Fig. 3.** (A) S1 mapping of RNA transcribed from the *Hin*III-K fragment during the early phase of infection.
Poly(A)$^+$ RNA obtained from the cells infected for 12 hr was S1-mapped using the right-hand *Eco*RV-*Hin*dIII subfragment of the *Hin*dIII-K region (5′ end-labelled at the *Hin*dIII site) as the probe (lane a). The S1-resistant signals indicated by the arrows were expected for the α transcripts as described (11). Labeled markers in the lanes b-c included *Hae*III-restricted pBR322 DNA (lane b) and φX174 DNA (lane c), respectively. (B) Transcription of the genes in the *Hin*dIII-K fragment in whole cell extract. Whole cell extract was prepared from uninfected *S. frugiperda* cells, and the *in vitro* transcription reactions with the whole cell extract were performed in the absence (lane NC) or presence (lane WX) of the *Hin*dIII-K fragment of the AcNPV genome. The DNA fragments were added as template for *in vitro* transcription after digestion with *Hin*dIII for linearization. *In vitro* synthesized RNAs are indicated by the solid bars at right (IV) which are expected to be corresponding to the transcripts in the panel A by the same order. Labelled markers in the lane M's contained λ DNA restricted with *Hin*dIII, and φX174 DNA digested with *Hae*III (from left to right), respectively. However, the size of the band at the middle of the left lane in the M's was estimated to be 564 nt.

activity (panel C). This observation strongly indicates that the novel RNA polymerase is related to early gene products of the virus because its presence is observed as early as 6 hr p.i., the time point at which the viral late genes are not yet transcribed.

***In vitro* Run-Off Transcription Analysis with Whole Cell Extract**

Friesen & Miller (4, 6) as well as Erlandson & Carstens (2) have mapped a set of overlapping transcripts in the *Hin*dIII-K/Q region of the AcNPV genome. Their S1 mapping and Northern blot analyses identified at least two early (α) transcripts from that region as early as 2 hr after infection which are transcribed rightward. Their 5′ ends lie about 430 bp, and 830 bp to the left of the *Hin*dIII K/Q boundary, respectively (Fig. 1 and 3A). These two rightward transcripts cross the *Hin*dIII K/Q boundary, and terminate the 3′ site at 540 bp downstream from the *Hin*dIII K/Q junction. Huh and Weaver (11) have also identified two other rightward transcripts, whose 5′ ends lie about 530, 670 and 680 bp to the left of the *Hin*dIII K/Q boundary, respectively (Fig. 3A). Two of them, whose 5′ ends mapped to 670 and 680 bp to the left of the *Hin*dIII K/Q boundary, respectively, were estimated to be early transcripts.

*In vitro* transcription analysis with whole cell extract was performed in the presence of α-$^{32}$P-GTP and as template the excised *Hin*dIII-K fragment with the *Hin*dIII restriction endonulease for linearization. Labeled RNA products synthesized by run-off reactions were analyzed by electrophoresis on polyacrylamide-urea sequencing gels followed by autoradiography for identifying putative 5′ ends of the α-gene transcripts (Fig. 3B). The incubation of the *Hin*dIII-K fragment with whole cell extract resulted in the synthesis of 4 major transcripts designated IV-1, IV-2, IV-3, and IV-4 whose sizes were 860, 680, 670, and 430 bp, respectively (Fig. 3B, lane WX). No significant transcript was made in the absence of the template DNA (lane NC). However, migration of RNA in the upper part of the polyacrylamide gel is frequently a little slower than that of similar-sized DNA fragments used for size markers. Hence, the length of the IV-1 was calculated to be about 830 bp, which corresponded to that of the $\alpha_2$ RNA described in Fig. 1. A doublet RNA consisting of IV-2 and IV-3 also corre-

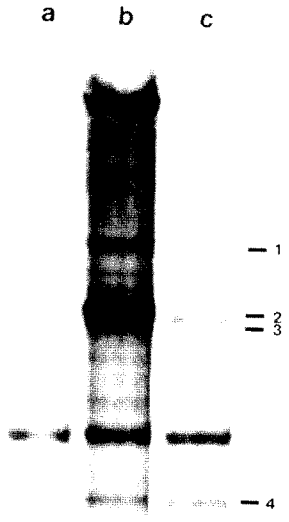


**Fig. 4.** *In vitro* transcription in nuclear extract.
Nuclear extract was prepared from uninfected cells as described in Methods (3). *In vitro* run-off transcription reaction was performed with 12.5 μl (lanes a~b) or 25 μl (lane c) of the nuclear extract in a 50 μl volume. The reactions were carried out in the absence (lane a) or presence (lanes b~c) of the *Hin*dIII-K fragment of the AcNPV genome. The DNA fragment was cleaved with *Hin*dIII for using as template in the *in vitro* reaction. The in vitro synthesized RNAs were indicated at right, and the RNA bands were the signals expected for the various transcripts in the *Hin*dIII-K region as follows, from top to bottom: the α-2 transcript (band 1 at 830 bp in Fig. 1); the two transcripts detected as trace amounts *in vivo* (bands 2 and 3 at about 670 bp in Fig. 3A); and the α-1 transcript (band 4 at 430 bp in Fig. 1).

sponded to *in vivo* RNAs whose S1 mapping signals showed 670, and 680 bp in size, respectively (Fig. 3A). However, the intensity of the *in vitro* synthesized RNAs in whole cell extracts were exactly reverse to that of *in vivo* RNAs (4, 11).

### *In vitro* Run-off Assays with Nuclear Extracts

*In vitro* run-off transcription assays with nuclear extracts were also carried out under various conditions for optimizing the reactions. The reactions included the linearized *Hin*dIII-K fragment for template from which several α transcripts were synthesized (Fig. 1, and 3A). The nuclear extracts were prepared from uninfected cells by the procedure described in Methods (3). When the incubation was performed under the standard conditions as described (3) with 12.5 μl of the nuclear extract in a 50 μl volume, the synthesis of RNAs (Fig. 4, lane b) closely paralleled the patterns observed with the whole cell extracts (Fig. 3B, lane WX) in accuracy of transcription initiation and abundance of RNA synthesis. However, when the reaction was carried out with 25 μl of the nuclear extract in a 50 μl reaction volume, the synthesis of various RNAs was reduced dramatically (lane c of Fig. 4).

Our earlier studies left some questions about whether α genes located in the *Hin*dIII-K/Q fragment of the viral genome were immediate early genes which were transcribed solely by host transcription machinery. According to this study, the protein factors in host cells were capable of accurately initiating the transcription from the α genes in the *Hin*dIII-K/Q region. However, the abundance of RNA syntheses in the nuclear extracts as well as in the whole cell extracts was reverse to that of *in vivo* RNA (Fig.s 3B and 4). Therefore, a factor(s) involved in regulating transcription intensity of genes was estimated to be missed in both the cell free extracts used in the experiments. The factor(s) might be either protein factor(s), sequence element(s), or both. If the protein factor(s) has been missed from the cell-free extracts prepared from uninfected cells, the α genes can be defined to be delayed early genes which transcription requires an additional protein(s) encoded from the viral gene(s) of AcNPV together with host protein factors. However, we can not rule out the possibility that in the cell-free extracts prepared under the conditions described above, the transcription factor(s) derived from host cells, and involved in regulating transcription efficiency of the α genes of the viral genome is missed during preparation of the extract.

### RNase Mapping of the p26 Transcript Labeled by *in vitro* Run-on Assay

Transcription initiation sites of several genes were mapped in the *Hin*dIII-Q region of the AcNPV genome (Fig. 1). Two of them, 1,500 and 1,100 bp long, first appeared at 6 hr p.i. and were presented in the greatest amount at 12 hr p.i. However, only trace amount of them remained at 18 hr p.i. These two transcripts contain an open reading frame that potentially encodes a 240 amino acid polypeptide of 26,000 dalton molecular weight (p26) (13, 17).

Transcription of the p26 gene in isolated nuclei appears to be inhibited by α-amanitin, which is characteristic of early transcripts in this virus (10). In order to verify this observation further, *in vitro* labeled RNA by run-on reactions in isolated nuclei was analyzed by the RNase mapping technique described in Methods (16). Briefly, labeled run-on RNA was isolated and hybridized to a molar excess of unlabeled antisense RNA probe specific for the p26 transcription region. This strand-specific RNA probe was synthesized by SP6 RNA polymerase from the *EcoRV-SalI* subfragment (656 bp) of the *Hin*dIII-Q region (Fig. 1), which was cloned into the

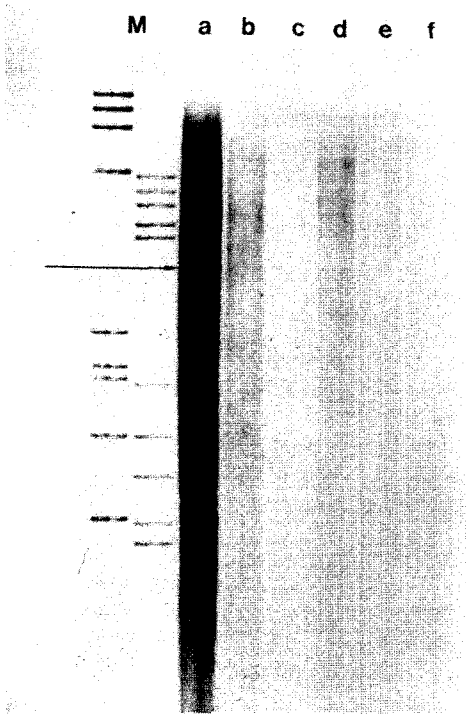


**Fig. 5.** RNase mapping of the p26 transcript.
RNA was labeled by *in vitro* run-on transcription in nuclei isolated at 12 hr (lanes a, b, and e) or 18 hr (lanes c, d, and f) postinfection. The reactions were run in the absence (lanes a, c, and e) or presence (lanes b, d, and f) of 2 μg/ml α-amanitin. The labeled RNAs were mapped with RNases as described in Methods (16) except that the RNAs were hybridized to unlabeled antisense RNA probes (corresponding to the *Eco*RV-*Sal*I subfragment of the *Hin*dIII-Q region of the AcNPV genome in Fig. 1) specific for p26 gene (lanes a~d), or incubated without the probes as control (lanes e~f). The band indicated by the arrow at 370 nt is the signal expected for the p26 transcript. Markers (lane M's) contained φX174 and pBR322 DNA digested with *Hae*III (from left to right), respectively.

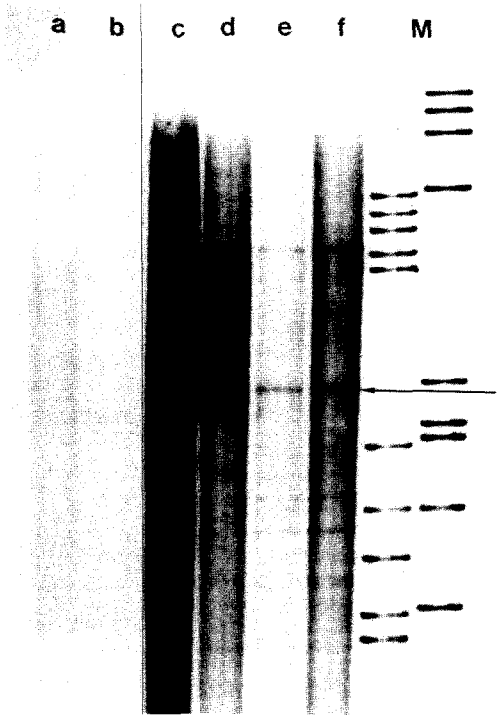


**Fig. 6.** RNase mapping of the p10 transcription during the course of infection.
RNA was labeled by *in vitro* run-on transcription in nuclei isolated at either 12 hr (lanes a, c, and d) or 18 hr (lanes b, e, and f) postinfection. The reactions were performed with the nuclei in the absence (lanes a, b, c, and e) or presence (lanes d, f) of 2 μg/ml α-amanitin. The labeled RNAs were hybridized to the cold strand-specific RNA probes for the p10 transcription region (from the *Xho*I-*Hin*dIII subfragment of the *Hin*dIII-Q as described in Fig. 1). As a control, RNase mapping was also carried out in the absence of the probes (lanes a~b). The band indicated by the arrow (304 nt) was expected for the p10 transcript (11,17). Markers in lanes Ms were the same ones used in Fig. 5 but in reverse order.

corresponding sites of pGEM-5zf(+) vector (Promega). The RNA fragments protected against RNase digestion were subjected to electrophoresis and autoradiography.

This analysis revealed two major RNA signals at 370 nucleotides (nt) and 656 nt, respectively (Fig. 5). The lower RNA band (arrowed) was the signal expected for the p26 transcript. The band at 656 nt, however, appeared to be consistent in size with the antisense RNA which was used for the probe. At 12 hr p.i. when accumulation of the RNA coding for the 26 kd protein (p26) was most prominent *in vivo*, a significant level of the signal for p26 RNA was detected at the correct position in the absence of α-amanitin (Fig. 5, lane a), while there was no significant signal observed in the presence of α-amanitin (lane b). However, by 18 hr p.i. no signal for the gene was obtained either in the absence (lane c) or in the presence (lane d) of α-amanitin, consistent with the data in the previous reports (11, 17). These observations suggested that transcription of the p26 gene was carried out by α-amanitin-sensitive host RNA polymerase II at least as late as 12 hr p.i. This experiment also suggests that at 18 hr p.i. there was no *de novo* synthesis of p26 mRNA, although trace amounts of the transcripts were still detected in the *S. frugiperda* cells infected with AcNPV (17). These results clearly demonstrate that the p26 gene is the early gene of AcNPV, which is transcribed by host cell-specific RNA polymerase II.

### RNase Mapping of p10 Transcripts Labeled by *in vitro* Run-on Reaction

Expression of the p10 gene, whose 5′ end maps 303 bp upstream from the *Hin*dIII-Q/P boundary (Fig. 1), is very late in the virus infection cycle. Its transcripts are detected first at 10 hr p.i. and most abundant at 24 hr p.i. (17). In order to investigate whether transcription of the p10 gene is performed by the novel, α-amanitin-resistant RNA polymerase, we also employed the RNase mapping technique described above. According

to the RNase mapping analysis shown in Fig. 6, by 18 hr p.i. *in vitro* run-on RNA specific for the p10 gene was synthesized regardless of whether the assay was carried out in the presence or absence of α-amanitin (lanes e, f). At 12 hr p.i., however, the signal was barely detectable in both reactions (lanes c, d). These observations firmly verified the previous conclusion that RNA coding for the p10 protein was synthesized by the virus-induced, α-amanitin-resistant RNA polymerase. Furthermore, the transcription pattern of the p10 gene in nuclei at two different time points (12 and 18 hr p.i.) was consistent with the previous reports concerning that the amount of *in vivo* transcripts of p10 gene at 12 hr p.i. are not significant in comparison with that observed at 18 hr p.i. (13, 17).

The data described in Fig. 5 and 6 confirm more directly than those in the previous reports (10, 117) that the p26 gene is an early one, and its transcription is directed by host RNA polymerase II but continues into the late phase of infection. On the other hand, the p10 gene, as expected, is a late one whose transcription is always α-amanitin-resistant.

The background signals observed in Fig. 5 and 6 are due to the presence of the transcripts overlapped with the p26 or p10 RNAs synthesized in nuclei which are also hybridizable to the antisense RNA added as probe in the RNase mapping analyses. The background signals are also due to the presence of transcripts synthesized bi-directionally from the other part of viral genome which are hybridizable from each other. However, at 12 h p.i. at which the viral transcription by RNA polymerase II still goes on, the non-specific background signals were decreased in the presence of α-amanitin. On the other hand, at 18 h p.i. at which most of the viral transcriptions were carried out by the α-amanitin-resistant RNA polymerase, the background was rather increased in the presence of α-amanitin. It may be due to the fact that at the early phase of viral infection transcription of the viral genome is dependent upon host RNA polymerase II, which is comletely inhibited by α-amanitin resulting in the reduction of RNA complimentary to each other. During the late phase of infection, however, most of the viral transcription is performed by the novel, α-amanitin-resistant RNA polymerase which may be rather activated by the inhibitor, consequently the increase in the synthesis of self-hybridizable RNA.

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