JOURNAL OF VIROLOGY, June 1981, p. 916-921
0022-538X/81/060916-06$02.00/0

Vol. 38, No. 3

α-Amanitin-Resistant Viral RNA Synthesis in Nuclei Isolated from Nuclear Polyhedrosis Virus-Infected *Heliothis zea* Larvae and *Spodoptera frugiperda* Cells

MARJORI A. GRULA,† PATRICIA L. BULLER, AND ROBERT F. WEAVER*

Department of Biochemistry, Smissman Research Laboratories, University of Kansas, Lawrence, Kansas 66045

Received 24 October 1980/Accepted 6 March 1981

[^{3}H]RNA was synthesized in nuclei isolated at various times postinfection from the fat bodies of *Heliothis zea* larvae infected with *H. zea* nuclear polyhedrosis virus and from cultured *Spodoptera frugiperda* cells infected with *Autographa californica* nuclear polyhedrosis virus. To detect virus-specific RNA synthesis, the [^{3}H]RNA was hybridized to denatured viral DNA immobilized on nitrocellulose filters. Nuclear polyhedrosis virus-specific RNA synthesis in the infected nuclei isolated from *H. zea* larval fat bodies and *S. frugiperda* cells was only inhibited 20 to 25% by concentrations of α-amanitin sufficient to inhibit the host RNA polymerase II. In addition, a productive nuclear polyhedrosis virus infection was obtained in *S. frugiperda* cells grown in the presence of an α-amanitin concentration that inhibited 90% of the cellular RNA polymerase II activity. The cellular RNA polymerase II enzyme remained sensitive to α-amanitin during infection, and there was no evidence that a virus-coded, α-amanitin-resistant enzyme was synthesized after the onset of infection. The data suggest that the bulk of nuclear polyhedrosis virus-specific RNA synthesis in isolated nuclei is transcribed by an enzyme other than the host RNA polymerase II.

Nuclear polyhedrosis viruses (NPVs) are insect pathogens that develop in the nuclei of infected cells, where they are occluded within crystalline protein structures known as polyhedral inclusion bodies. NPVs have double-stranded circular DNA genomes with molecular weights ranging from 80 to more than 90×10^6 (6, 12). Very little is known about the synthesis of viral RNA from this DNA template during infection. Since no endogenous RNA polymerase activity associated with either the occluded or nonoccluded forms of the virus can be detected (Grula, unpublished results), it was hypothesized that a host RNA polymerase enzyme(s) is responsible for transcribing viral RNA, at least early in infection. It has been shown for a number of animal nuclear DNA viruses, such as adenovirus 2 (8, 15), adenovirus-associated virus (2), herpes simplex virus type 1 (1), bovine parvovirus (7), and simian virus 40 (5), that the host RNA polymerase II, normally responsible for cellular mRNA synthesis, transcribes most or all of the viral RNA. For the studies presented in this paper, nuclei isolated from *Heliothis zea* NPV (HzNPV)-infected *H. zea* larval fat bodies and *Autographa californica* NPV (AcNPV)-infected *Spodoptera frugiperda* cells have been used to study the role of the host RNA polymerases during NPV infection. The results obtained indicated that 75 to 80% of the NPV-specific RNA synthesis in nuclei isolated from both sources was resistant to inhibition by α-amanitin, a potent inhibitor of eucaryotic RNA polymerase II.

† Present address: Department of Biology, Washington University, St. Louis, MO 63130.

MATERIALS AND METHODS

Biochemicals. Calf thymus DNA (type 1), penicillin G, and streptomycin sulfate were purchased from Sigma Chemical Co., St. Louis, Mo. Unlabeled nucleoside triphosphates were obtained from P-L Biochemicals Inc., Milwaukee, Wis. α-Amanitin was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. [5-^{3}H]UTP, tetrasodium salt (10 to 25 Ci/mmol), was purchased from Schwarz/Mann, Spring Valley, N.Y. Phenol (liquified) was purchased from Fisher Scientific Co., Fairlawn, N.J. The phenol was distilled before use and stored in dark bottles at −20°C. All other chemicals were of reagent grade and were used without further purification.

Growth of *H. zea* larvae. Laboratory stocks of *H. zea* were reared on an artificial diet as described elsewhere (Grula and Weaver, Insect Biochem., in press).

Infection of *H. zea* larvae with HzNPV. Fourth instar larvae, weighing between 100 and 200 mg, were infected by surface contamination of their food with

2.5×10^4 HzNPV inclusion bodies per cm^2. When infected in this way, 90 to 100% of the larvae died on day 7 postinfection. The isolation of HzNPV inclusion bodies and HzNPV DNA was performed as described previously (11).

Isolation of *H. zea* fat body nuclei. Fat body tissue was removed from *H. zea* larvae, and the nuclei were isolated by the procedure of Elshourbagy and Wilkinson (4) for isolation of nuclei from *Spodoptera eridania* larval midgut tissue. The nuclei thus obtained were stored at −80°C in the following buffer (TGED): 50 mM Tris-hydrochloride (pH 7.9), 35% glycerol, 0.1 mM EDTA, and 0.5 mM dithiothreitol. The final concentration of nuclei was approximately 10^7/ml.

Growth and infection of *S. frugiperda* cells. A continuous cell line of *S. frugiperda* cells (IPLB-SF-21) was obtained from Lois K. Miller (University of Idaho). The cells were cultured at 27°C in TC-100 basal medium (M.A. Bioproducts, Walkersville, Md.) supplemented with 0.26% tryptose broth (Difco Laboratories, Detroit, Mich.), 8% fetal calf serum (M.A. Bioproducts), 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

The cells were infected with nonoccluded virions of AcNPV obtained from the hemolymph of infected *S. frugiperda* larvae or early passage culture medium from infected *S. frugiperda* cells. Both infectious medium and infectious hemolymph (diluted in TC-100 medium) were sterilized by filtration through a membrane filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.). Virus titers were adjusted such that the multiplicity of infection was 10 to 20 50% tissue culture infective doses per cell.

Isolation of *S. frugiperda* cell nuclei. All procedures for isolation of cell nuclei of *S. frugiperda* were carried out at 0 to 4°C. After removal of the culture medium, the cells were suspended in 10 ml of phosphate-buffered saline (0.14 M NaCl, 2.6 mM KCl, 10.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.9 mM $CaCl_2$) by pipetting up and down with a Pasteur pipette. The cells were pelleted by centrifuging at $350 \times g$ for 5 min. The cell pellet was suspended in 10 ml of hypotonic buffer (10 mM Tris-hydrochloride [pH 7.9], 10 mM NaCl, 1.5 mM magnesium acetate) and allowed to swell on ice for 10 to 15 min. After this, Triton X-100 was added to make a 0.5% solution, and the cells were transferred to a Dounce homogenizer (Wheaton Scientific Products, Millville, N.J.) and homogenized with 10 passes of a type A rod. The nuclei were pelleted by centrifuging at $1,000 \times g$ for 5 min. The pelleted nuclei were suspended in TGED at a concentration of 10^7 nuclei per ml, quick-frozen in a dry ice-ethanol bath, and stored at −80°C.

RNA synthesis in isolated nuclei. RNA synthesis reactions were carried out in a mixture containing 50 mM Tris-hydrochloride (pH 7.9); 0.9 mM each GTP, ATP, and CTP; 0.6 mM NaF; 1.6 mM $MnCl_2$; 0.02% β-mercaptoethanol; 100 mM $(NH_4)_2SO_4$; 16 μM UTP; 1 μCi of [^{3}H]UTP (18 Ci/mmol); and 2×10^5 nuclei per ml in a total volume of 60 μl. The reaction was initiated by the addition of the isolated nuclei to the other assay components. In experiments involving α-amanitin, nuclei were added to an assay mixture that already contained the toxin. After incubation for 10 min, a 40-μl sample was removed and spotted on a DE81 disk (Whatman, Inc., Clifton, N.J.). The disks were washed and counted for radioactivity as described elsewhere (Grula and Weaver, in press).

To synthesize [^{3}H]RNA for hybridization experiments, the above procedure was followed, except the reaction volume was increased to 330 μl, and 22 μCi of [^{3}H]UTP was used. The reaction was carried out in 1.5-ml Eppendorf polypropylene tubes (Brinkmann Instruments, Inc., Westbury, N.Y.). All tubes, pipette tips, and solutions were sterilized by autoclaving. After 10 min of incubation, an equal volume of 10 mM sodium acetate–10 mM EDTA (pH 5.1) buffer and 0.1 volume of 10% sodium dodecyl sulfate were added to the reaction mixture. An equal volume of water-saturated phenol equilibrated to 60°C was then added and blended in a Vortex mixer with the reaction mixture. The tubes were incubated at 60°C for 5 min with intermittent blending and then centrifuged in an Eppendorf microcentrifuge (Brinkmann) to separate the phenol and aqueous phases. The aqueous phase was removed, and 0.1 volume of 3 M sodium acetate (pH 5.2) was added, followed by 2 volumes of cold 95% ethanol. The ethanol suspension was stored overnight at −20°C. The precipitated RNA was collected by centrifugation in an Eppendorf microcentrifuge and dried in a Nalgene vacuum desiccator (Nalge/Sybron Corp., Rochester, N.Y.). The RNA isolated in this manner (low pH, high temperature) was shown to be sensitive to degradation by RNase and free from contamination by DNA as measured by the diphenylamine assay (13) (data not shown). The RNA concentration was determined by measuring the absorbance at 260 nm.

Isolation of AcNPV DNA. AcNPV DNA was isolated from inclusion bodies as described previously (6).

Preparation of NPV DNA-containing nitrocellulose filters. Nitrocellulose filters containing 2 μg of NPV DNA or 2 μg of calf thymus DNA were prepared as described by Raskas and Green (9). Nitrocellulose filters (0.45-μm pore size) were obtained from Gelman Instrument Co., Ann Arbor, Mich.

Hybridization reactions. The dried, ethanol-precipitated [^{3}H]RNA synthesized in isolated nuclei was resuspended in 1,350 μl of sterile hybridization buffer (2× SSC, 0.5% sodium dodecyl sulfate; 1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), boiled for 3 min, and quenched in an ice bath. Samples of 250 μl were transferred to small glass scintillation vials containing the DNA filters. Triplicate filters of viral DNA were run for each RNA sample, as well as one calf thymus DNA filter and one blank filter to measure nonspecific binding and background, respectively. To determine input counts of [^{3}H]RNA, 50 μl of each RNA solution was spotted on a DE81 disk and washed and counted for radioactivity as described elsewhere (Grula and Weaver, in press). To insure that viral DNA was present in excess in the hybridization reactions, higher concentrations of RNA were tested, and it was shown that these gave proportionally higher hybridization (data not shown). The vials were tightly capped and placed in a 50°C water bath for 18 to 20 h. After hybridization, the remaining solution was removed with a Pasteur pipette. The filters were washed

for 10 min at 50°C in 2 ml of hybridization buffer and then two times for 30 min with 2× SSC. After the final wash, the filters were transferred to new scintillation vials and dried for 2 h in vacuo at 80°C. Two milliliters of Omnifluor (4 g/liter; New England Nuclear Corp., Boston, Mass.) in toluene was added to the vials, and the filters were counted for radioactivity. The efficiency of hybridization with this technique was determined to be 54% (data not shown).

Inhibition of endogenous RNA polymerase II in *S. frugiperda* cells by the addition of α-amanitin to the cell culture medium. *S. frugiperda* cells were treated with α-amanitin by adding the toxin directly to nonconfluent cells growing in fresh culture medium. After 18 h, the nuclei were harvested and used to synthesize RNA in vitro in the presence and absence of 15 μg of α-amanitin per ml. The percent inhibition of nuclear RNA synthesis by α-amanitin in the reaction mixture was taken to be a measure of the remaining endogenous RNA polymerase II activity in the nuclei.

Isolation of *S. frugiperda* RNA polymerase activities. RNA polymerase activities were solubilized from *S. frugiperda* cells and chromatographed on DEAE-Sephadex (Sigma) by a procedure described elsewhere for *H. zea* RNA polymerase (Grula and Weaver, in press). The fractions eluting from the DEAE-Sephadex column were assayed for RNA polymerase activity as described elsewhere (Grula and Weaver, in press).

RESULTS

For the studies reported in this paper, it was important to show that α-amanitin entered the isolated nuclei and inhibited the activity of RNA polymerase II. The presence of α-amanitin in the in vitro reaction mixture caused a reduction of total RNA synthesis in nuclei isolated from *H. zea* larval fat bodies (Fig. 1). Maximal inhibition (30%) was observed at 5 μg of α-amanitin per ml. This reduction of RNA synthetic activity at a low concentration of α-amanitin was due to inhibition of RNA polymerase II since the *H. zea* RNA polymerases I and III are resistant to inhibition by α-amanitin (17; Grula and Weaver, in press).

To determine the extent to which NPV-specific RNA synthesis was inhibited by α-amanitin, nuclei were isolated from *H. zea* larval fat body tissue at 48, 72, 96, and 120 h postinfection and used to synthesize RNA in vitro in the presence and absence of 15 μg of α-amanitin per ml. [^{3}H]RNA was also synthesized in nuclei isolated from uninfected larvae at approximately the same developmental stage as the infected larvae. The [^{3}H]RNA was isolated and hybridized to HzNPV DNA-containing nitrocellulose filters (Fig. 2). NPV-specific RNA synthesis in isolated nuclei increased at 48 to 96 h postinfection and then began to level off. When RNA was synthesized in the presence of 15 μg of α-amanitin per

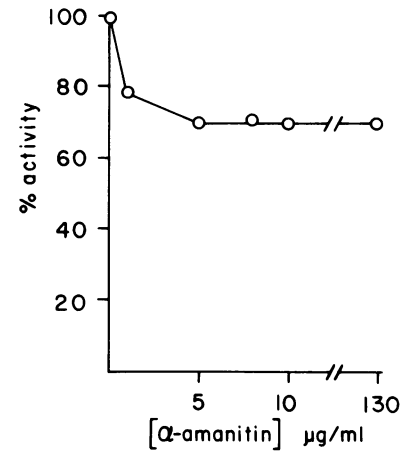

FIG. 1. *Inhibition by α-amanitin of total RNA synthesis in H. zea fat body nuclei. Nuclei were isolated and RNA synthesis reactions were carried out as described in the text in the presence of increasing concentrations of α-amanitin. The points represent the average of triplicate assays.*

ml, an amount that is in excess of the concentration required for maximal inhibition (Fig. 1), only 20 to 25% of the NPV-specific RNA synthesis was inhibited. A negligible amount of [^{3}H]-RNA synthesized in uninfected nuclei hybridized to NPV DNA, thus verifying that NPV-specific RNA is synthesized only in infected nuclei.

Since the infection in the *H. zea* larvae is difficult to control (different populations of larvae can vary in their susceptibility to infection), the same experiment was performed with nuclei isolated at 8, 16, and 24 h postinfection from *S. frugiperda* cells infected with AcNPV (Fig. 3). Again, approximately 75% of the NPV-specific RNA synthesized in the infected nuclei was resistant to inhibition by α-amanitin.

These results are unusual since they suggest that an RNA polymerase activity other than the host RNA polymerase II, which is inhibited by low concentrations of α-amanitin (17; Grula, unpublished observations), transcribes the bulk of the NPV-specific RNA. This suggestion was tested by monitoring the progress of AcNPV infection in *S. frugiperda* cells when α-amanitin was present in the culture medium (Table 1). A productive NPV infection (as determined by the appearance of inclusion bodies in the nuclei of infected cells) was obtained even when the cellular RNA polymerase II activity was inhibited 90% by 25 μg of α-amanitin per ml in the cell culture medium.

To determine whether the host RNA polymerase II activity, initially sensitive to α-amanitin, became resistant during infection, RNA polym-

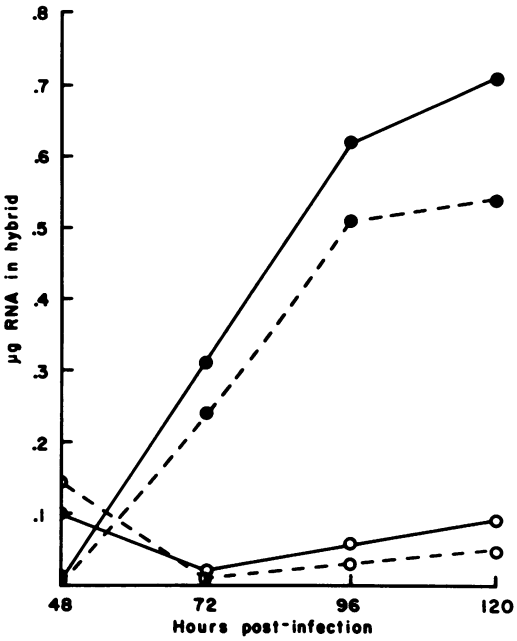

FIG. 2. *Synthesis of virus-specific RNA in isolated infected H. zea fat body nuclei. HzNPV DNA was hybridized to [^{3}H]RNA synthesized in the presence and absence of α-amanitin in H. zea fat body nuclei isolated at the indicated times postinfection and fat body nuclei isolated from uninfected larvae at approximately the same developmental stage as the infected larvae. Data are corrected for nonspecific hybridization of the [^{3}H]RNA samples to calf thymus DNA. The hybridization results shown are representative of an experiment repeated twice. [^{3}H]RNA was synthesized in isolated nuclei and hybridized to NPV DNA as described in the text. Symbols: ●——●, RNA synthesized in infected nuclei minus α-amanitin; ●---●, RNA synthesized in infected nuclei in the presence of 15 μg of α-amanitin per ml; ○——○, RNA synthesized in uninfected nuclei minus α-amanitin; ○---○, RNA synthesized in uninfected nuclei plus 15 μg of α-amanitin per ml.*

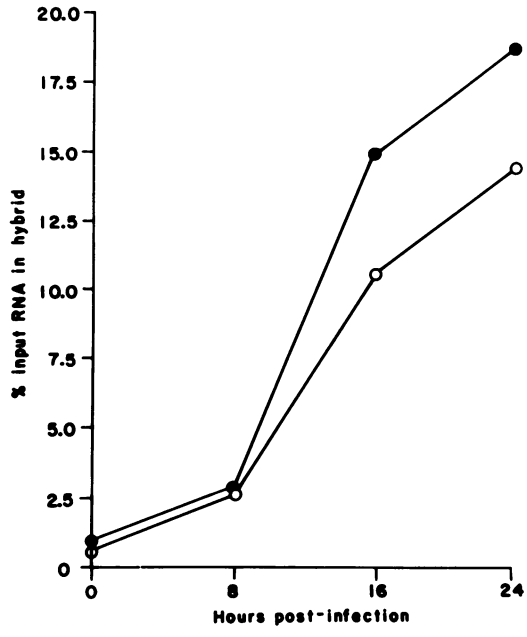

FIG. 3. *Synthesis of virus-specific RNA in isolated, infected S. frugiperda cell nuclei. AcNPV DNA was hybridized to [^{3}H]RNA synthesized in the presence (○) and absence (●) of 15 μg of α-amanitin per ml in S. frugiperda cell nuclei isolated at the indicated times postinfection. RNA synthesis reactions and hybridizations were performed as described in the text. Data are corrected for nonspecific hybridization of the [^{3}H]RNA samples to calf thymus DNA. The hybridization results shown are representative of an experiment repeated three times.*

erase activities were solubilized from uninfected *S. frugiperda* cells and from *S. frugiperda* cells 16 h postinfection. The solubilized enzymes were chromatographed on DEAE-Sephadex and eluted individually with an increasing concentration of ammonium sulfate. Fractions collected from the column were assayed for RNA polymerase activity in the presence and absence of 1 μg of α-amanitin per ml. The DEAE-Sephadex elution profiles of RNA polymerase activities were virtually identical from infected and uninfected cells, and the solubilized host RNA polymerase II maintained its original sensitivity to α-amanitin during infection (Fig. 4). (Peaks are identified in accord with previous studies on *H. zea* RNA polymerase [Grula and Weaver, in press].) In addition, this experiment did not reveal any new, chromatographically distinct species of RNA polymerase which might corresond to a virally coded, α-amanitin-resistant enzyme

TABLE 1. *Inhibition of S. frugiperda cellular RNA polymerase II activity and NPV infection by various concentrations of α-amanitin in the cell culture medium*[a]

Concn of α-amanitin (μg/ml)	% Inhibition of RNA polymerase II	% of cells with polyhedra 48 h postinfection
0	0	100
5	65	95–100
15	73	80–85
25	90	>0[b]

[a] Cells were pretreated with α-amanitin for 18 h before they were inoculated with virus. The percent inhibition of RNA polymerase II was determined after this pretreatment as described in the text.

[b] The cells at the higher α-amanitin concentrations, particularly 25 μg/ml, were showing severe cytopathic effects by 48 h postinfection, and the number of infected cells was difficult to quantify.

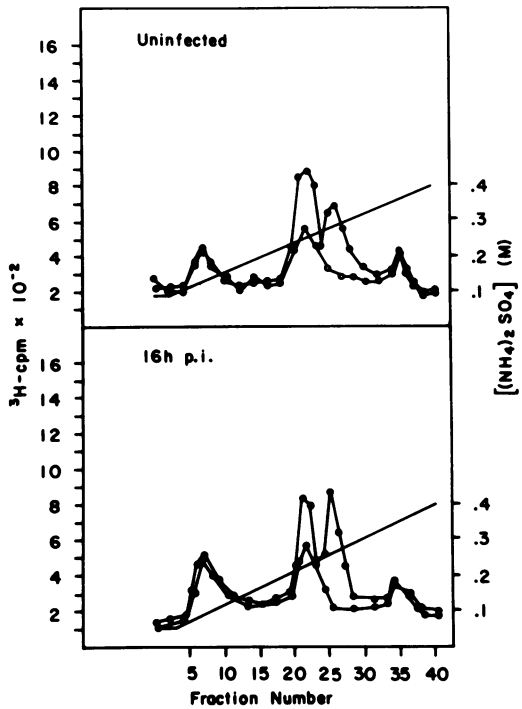

FIG. 4. *DEAE-Sephadex elution profiles and activities of S. frugiperda RNA polymerases from infected and uninfected cells in the presence and absence of 1 μg of α-amanitin per ml. RNA polymerase activities were solubilized from uninfected and infected (16 h postinfection) S. frugiperda cells by a procedure described elsewhere (Grula and Weaver, in press) and chromatographed on a 2.5-ml DEAE-Sephadex column. A linear gradient of 0.04 to 0.40 M ammonium sulfate was used to elute the enzyme activities. The fractions collected were assayed as described elsewhere (Grula and Weaver, in press) in the presence* (○) *and absence* (●) *of 1 μg of α-amanitin per ml.* (——), *Ammonium sulfate concentration.*

that is synthesized after the onset of infection.

To verify that the unsolubilized host RNA polymerase II also remained sensitive to α-amanitin during infection, the response of the endogenous RNA polymerase II to α-amanitin was tested in *S. frugiperda* nuclei isolated at various times postinfection. The percent inhibition of total RNA synthesis by 15 μg of α-amanitin per ml varied during infection, but at all times tested there was a reduction of total RNA synthesis in the isolated nuclei in the presence of α-amanitin (Table 2). This reduction reflected the endogenous RNA polymerase II activity in the nuclei and indicated that it was still sensitive to α-amanitin in infected cells.

To minimize DNA contamination of the RNA preparations in these studies, a low-pH, high-temperature phenol extraction procedure was employed. To rule out the possibility that this procedure selects against mRNA, extractions of labeled RNA from uninfected nuclei were carried out in parallel, using the low-pH, high-temperature procedure and the typical phenol-chloroform-isoamyl alcohol technique (3). The extracted RNAs were chromatographed on oligodeoxythymidylic acid-cellulose to determine the polyadenylated RNA contents. These were found to be very similar: of 8,250,000 cpm of RNA extracted by the phenol-chloroform-isoamyl alcohol technique, 76,000 cpm (0.9%) was polyadenylated; of 7,150,000 cpm of RNA extracted by the low-pH, high-temperature procedure, 59,000 cpm (0.8%) was polyadenylated.

TABLE 2. *Percent inhibition of total RNA synthesis by 15 μg of α-amanitin per ml in S. frugiperda nuclei isolated at various times postinfection*[a]

Time of nuclear isolation (h postinfection)	% Inhibition
0	26
8	40
16	13
24	16

[a] The results shown are the average of duplicate assays. Nuclei were isolated and the RNA synthesis reactions were performed as described in the text.

DISCUSSION

The results reported here demonstrate that a large fraction of NPV-specific RNA synthesized in isolated infected nuclei is not inhibited by concentrations of α-amanitin in excess of that required to inhibit the cellular RNA polymerase II. This result was unexpected since RNA polymerase II is the enzyme normally responsible for transcribing cellular mRNA and the mRNA for all nuclear-replicating DNA viruses thus far studied (1, 2, 5, 7, 8, 15). Furthermore, productive NPV infection was observed in *S. frugiperda* cells treated with α-amanitin before infection. Even though it could not be shown that 100% of the endogenous RNA polymerase II activity was inhibited, these results were considered significant and compatible with the hybridization data.

To determine whether the α-amanitin-resistant NPV RNA synthesis was due to a change in the response of the host RNA polymerase II to α-amanitin, RNA polymerase activities were solubilized from infected and uninfected *S. frugiperda* cells and chromatographed on DEAE-Sephadex. When the fractions eluting from the column were assayed for RNA polymerase activity in the presence and absence of α-amanitin, it was shown that the solubilized RNA polymerase II remained completely sensitive to α-amanitin during infection. The continued sensitivity to

α-amanitin of the cellular RNA polymerase II during infection was also observed when the endogenous RNA polymerase II activity was measured in isolated nuclei.

There is a possibility that the NPV genome codes for an RNA polymerase that is synthesized after the onset of infection, since only 15 to 20% of the coding capacity of the genome can be accounted for by viral structural proteins (14). However, no new, chromatographically distinct RNA polymerase activity was detected when the enzymes were solubilized from infected cells and chromatographed on DEAE-Sephadex. The possibility of a virus-coded enzyme cannot be entirely ruled out, though, since the isolation procedure used may not have been suitable for recovering a viral RNA polymerase.

A final explanation for the α-amanitin-resistant NPV RNA synthesis is that one of the host α-amanitin-resistant enzymes, either RNA polymerase I, RNA polymerase III, or both, transcribes the bulk of the viral RNA. It was not possible to test this hypothesis since both enzymes from these sources appear to be resistant to even high concentrations of α-amanitin (17; Grula and Weaver, in press; Grula, unpublished observations), and there are currently no other inhibitors available for either enzyme.

The only other case for a nuclear DNA virus genome being transcribed by a host RNA polymerase other than RNA polymerase II is during adenovirus 2 infection of KB cells (16). Here, a class of low-molecular-weight RNAs (VA RNAs) is transcribed by the host RNA polymerase III. This synthesis by RNA polymerase III comprises about 30% of the adenovirus-specific RNA synthesis in isolated, infected KB cell nuclei. In NPV-infected insect cells, by contrast, a large proportion (75 to 80%) of the NPV-specific RNA (presumably NPV mRNA) is synthesized by an α-amanitin-resistant enzyme. Based on this fact and the finding that a productive infection can be obtained in cells in the presence of α-amanitin, it appears that NPV RNA synthesis is due primarily to the activity of an RNA polymerase other than the host RNA polymerase II.

ACKNOWLEDGMENTS

We thank John Gaito and Bruce Underwood for their assistance in performing these experiments and Lois K. Miller, University of Idaho, for generous gifts of cells and virus and for advice in culturing them.

This work was supported by Public Health Service grant GM 22127 to R.F.W. from the National Institutes of Health and by grants from the General Research Fund, the Biomedical Science Support Grant Fund of the University of Kansas, and the Mid-America Cancer Center Program to R.F.W.

LITERATURE CITED

1. **Alwine, J. C., W. L. Steinhart, and C. W. Hill.** 1974. Transcription of herpes simplex type 1 DNA in nuclei isolated from infected HEp-2 and KB cells. Virology **60**:302-307.
2. **Bloom, M. E., and J. A. Rose.** 1978. Transcription of adenovirus-associated virus RNA in isolated KB cell nuclei. Virology **84**:118-126.
3. **Efstratiadis, A., and F. C. Kafatos.** 1976. The chorion of insects: techniques and perspectives, p. 1-124. *In* J. Last (ed.), Eukaryotes at the subcellular level. Marcel Dekker, Inc., New York.
4. **Elshourbagy, N. A., and C. F. Wilkinson.** 1978. An improved method for the isolation of intact nuclei from southern army worm (*Spodoptera eridania*) larval midgut. Insect Biochem. **8**:417-423.
5. **Jackson, A. H., and B. Sugden.** 1972. Inhibition by α-amanitin of simian virus 40-specific ribonucleic acid synthesis in nuclei of infected monkey cells. J. Virol. **10**: 1086-1089.
6. **Miller, L. K., and K. P. Dawes.** 1979. Physical map of the DNA genome of *Autographa californica* nuclear polyhedrosis virus. J. Virol. **29**:1044-1055.
7. **Patton, J. T., E. R. Stout, and R. C. Bates.** 1979. Transcription of the bovine parvovirus genome in isolated nuclei. J. Virol. **30**:917-922.
8. **Price, R., and S. Penman.** 1972. Transcription of adenovirus genome by an α-amanitin-sensitive ribonucleic acid polymerase in HeLa cells. J. Virol. **9**:621-626.
9. **Raskas, H. J., and M. Green.** 1971. DNA-RNA and DNA-DNA hybridization in virus research, p. 247-269. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol. 5. Academic Press, Inc., New York.
10. **Roeder, R. G.** 1976. Eukaryotic nuclear RNA polymerases, p. 285-329. *In* R. Losic and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Scharnhorst, D. W., K. L. Saving, S. Vuturo, P. H. Cooke, and R. F. Weaver.** 1977. Structural studies on the polyhedral inclusion bodies, virions, and DNA of the nuclear polyhedrosis virus of the cotton bollworm *Heliothis zea*. J. Virol. **21**:292-300.
12. **Scharnhorst, D. W., and R. F. Weaver.** 1979. Characterization of the DNA of the nuclear polyhedrosis virus of the cotton bollworm, *Heliothis zea*. J. Gen. Virol. **42**: 633-636.
13. **Shatkin, A.** 1969. *In* K. Habel and N. Salzman (ed.), Fundamental techniques in virology, p. 231. Academic Press, Inc., New York.
14. **Summers, M. D., and G. E. Smith.** 1978. Baculovirus structural polypeptides. Virology **84**:390-402.
15. **Wallace, R. D., and J. Kates.** 1972. State of adenovirus 2 deoxyribonucleic acid in the nucleus and its mode of transcription: studies with isolated viral deoxyribonucleic acid-protein complexes and isolated nuclei. J. Virol. **9**:627-635.
16. **Weinmann, R., T. G. Brendler, H. J. Raskas, and R. G. Roeder.** 1976. Low molecular weight viral RNAs transcribed by RNA polymerase III during adenovirus 2 infection. Cell **7**:557-566.
17. **West, T. W., M. A. Grula, W. M. Wormington, and R. F. Weaver.** 1980. Isolation of three forms of DNA-dependent RNA polymerase from *Heliothis zea* fat body tissue. Insect Biochem. **10**:509-513.