Time Course Analysis and Mapping of *Autographa californica* Nuclear Polyhedrosis Virus Transcripts

DENNIS Z. ROHEL AND PETER FAULKNER*

Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, K7L 3N6 Canada

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To study the expression of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) genome, intracellular virus-specific proteins and mRNAs were pulsed-labeled, extracted, and analyzed at 6-h intervals during the replicative cycle. Most RNAs were detected between 12 and 24 h postinfection (p.i.), but many continued to be synthesized until late in infection. Polyhedrin and p8 mRNAs were the two most abundant late viral RNA transcripts, and they were synthesized at high rates until late in the infection cycle (60 h p.i.). The abundance control of polyhedrin and p8 polypeptides was considered to be at the level of transcription. Two other major mRNAs in infected cells were 0.6-kilobase RNA, which was synthesized at its highest rate 12 to 18 h p.i., and 2.8-kilobase RNA, which was synthesized from 12 h p.i. until 48 h p.i. Cytoplasmic polyadenylic acid-containing RNA was isolated at 6-h intervals and was analyzed by Northern blot hybridization. At least 50 virus RNA transcripts were recognized, sized, and mapped onto the genome. Six RNAs hybridized to *EcoRI*-H, -I, and -J, and *HindIII*-Q AcNPV DNA restriction fragments, seven RNAs hybridized to *EcoRI*-B and -D DNA fragments, five RNAs hybridized to *EcoRI*-A and -E regions of the genome, four RNAs hybridized to *EcoRI*-C and -N DNA fragments, and one RNA species hybridized to *EcoRI*-O AcNPV DNA. A transcription map of the AcNPV genome was constructed, and the data were correlated with previously published translation maps.

The *Autographa californica* nuclear polyhedrosis virus (AcNPV) genome is a closed circular double-stranded DNA having a molecular weight of about 85×10^6 (130 kilobases [kb]) (17, 21) and is considered large enough to code for 75 or more proteins (22). Nonoccluded extracellular virus consists of 18 to 35 polypeptides, as determined by one-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (6, 9, 16, 26, 29), and 81 polypeptides have been resolved by two-dimensional gel electrophoresis of AcNPV occluded virus (20). Several investigators have detected between 30 and 36 infected cell-specific proteins (ICSPs) in pulse-labeled, AcNPV-infected cells (6, 9, 16, 29). ICSP synthesis is considered to be under temporal control, and the infection cycle has been divided into three (9) or four (14, 29) principal phases. The induction of a later phase of ICSP synthesis required the preceding phase of viral ICSP synthesis to be functional (14).

A detailed characterization of gene products (namely, RNA transcripts and polypeptides) is necessary in order to understand the organization of the large AcNPV genome. Several translation maps of AcNPV DNA have been published (1, 11, 22). Using hybridization selection followed by in vitro translation, Smith et al. (22) mapped 5 early and 19 late viral polypeptides, Esche et al. (11) mapped 4 early and 24 late polypeptides, and Adang and Miller (1) mapped 11 late viral gene products (see Fig. 7).

Little has been established about the nature and abundance of viral mRNA. Erlandson and Carstens (10) have shown that early transcription occurs at loci throughout the genome. Cytoplasmic RNA isolated 24 h postinfection (p.i.) hybridized with all AcNPV DNA fragments, suggesting that viral transcripts are derived from dispersed regions of the genome (27). However, so far only two major AcNPV transcripts have been isolated and translated in vitro (19, 23).

Polyhedrin mRNA (1.2 to 1.4 kb) hybridized to the *BamHI*-F and *HindIII*-V region of AcNPV DNA around map position 5%, and p8 mRNA (0.6 to 0.75 kb) hybridized to *EcoRI*-P and *HindIII*-Q fragments around map position 90% (19, 23).

In this paper we describe the approximate genomic locations of viral RNA transcripts present in the cytoplasm at times ranging from 6 to 66 h p.i. We show the sequential appearance of viral RNA transcripts, and we demonstrate that in the case of polyhedrin and p8 polypeptide both temporal control and protein abundance control are at the level of transcription.

MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* cells (25) were grown and maintained on BML-TC/10 medium (12) as previously described (19). Strain HR-3 of AcNPV (4) was used to infect *S. frugiperda* cells at a multiplicity of infection (MOI) of 10 PFU/cell.

Radiolabeling of cells. (i) RNA. Infected *S. frugiperda* cells (MOI, 10 PFU/cell) were pulse-labeled for 6 h at 6-h intervals with 30 μ Ci of [5,6- 3 H]uridine (42 Ci/mmol; New England Nuclear Corp., Boston, Mass.) per ml in BML-TC/10 medium containing no fetal bovine calf serum.

(ii) Proteins. ICSPs were labeled for 6 h at 6-h intervals with 30 μ Ci of [4,5- 3 H]leucine (59.8 Ci/mmol) per ml in L-leucine-free medium lacking fetal bovine calf serum. After labeling, the cells were washed with ice-cold BML-TC/10 medium, pelleted by centrifugation at $700 \times g$ for 5 min, suspended in electrophoresis sample buffer (5), heated in a boiling water bath for 5 min, and stored at -20°C .

Polyacrylamide gel analysis. The ICSPs were analyzed by discontinuous SDS-polyacrylamide slab gel electrophoresis (15). The stacking gel (5% acrylamide) contained 125 mM Tris-hydrochloride (pH 6.8), 0.1% SDS, and 2 mM EDTA, and the resolving gel (14% acrylamide) contained 400 mM Tris-hydrochloride (pH 8.8), 0.1% SDS, and 2 mM EDTA.

* Corresponding author.

Electrophoresis was at 60 V through the stacking gel and at 120 V through the resolving gel. After electrophoresis the gel was impregnated with EN³HANCE (New England Nuclear Corp.) essentially as recommended by the manufacturer. Radioactive proteins were detected by fluorography at -70°C , using Kodak XAR-5 X-ray film. Approximate molecular weights were determined from the migration distances of the ICSPs relative to the migration distances of the following prestained low-molecular-weight markers: ovalbumin (molecular weight, 43,000 [43K]), alpha-chymotrypsinogen (27.5K), beta-lactoglobulin (18.4K), lysozyme (14.3K), cytochrome *c* (12.3K), bovine trypsin inhibitor (6.2K), and insulin (3K); (Bethesda Research Laboratories, Inc., Bethesda, Md.).

RNA isolation. Infected cells were harvested at the end of each pulse-labeling period, washed with ice-cold BML-TC/10 medium, pelleted by centrifugation at $700 \times g$ for 5 min at 4°C , and lysed in a solution containing 30 mM Tris-hydrochloride (pH 8.3), 50 mM NaCl, 5 mM MgCl_2 , 250 mM sucrose, 200 μg of heparin per ml, and 0.5% Nonidet P-40 for 5 min at 0°C . Cytoplasmic RNA was extracted as described previously (19). Polyadenylic acid-containing [poly(A)⁺] RNA was selected by binding to oligodeoxythymidylic acid coupled to cellulose (Bethesda Research Laboratories), using the method of Aviv and Leder (3).

Methyl mercury gel electrophoresis. Cytoplasmic poly(A)⁺ RNA was denatured with 12 mM methyl mercury hydroxide (CH_3HgOH) for 20 min and fractionated by electrophoresis through 1% agarose gels containing 5 mM CH_3HgOH (19). The steps involving CH_3HgOH were done in a fumehood. After electrophoresis, the gel was detoxified by washing it for 40 min in either 0.5 M ammonium acetate or 5 mM 2-mercaptoethanol (gels used for Northern blot transfer) and then stained with ethidium bromide (2 $\mu\text{g}/\text{ml}$). Then gels were impregnated with EN³HANCE (New England Nuclear Corp.) and the RNA was detected by fluorography at -70°C , or the gels were used for transfer of fractionated RNA to diazobenzoyloxymethyl (DBM) paper.

Blot hybridization. Cytoplasmic poly(A)⁺ RNA that was fractionated according to size by electrophoresis under denaturing conditions was transferred to DBM paper (Schleicher & Schuell Inc., Keene, N.H.) by the method of Alwine et al. (2). Plasmid pBR322 DNAs containing single *EcoRI*-A, -B, -C, -D, -E, -H, -I, -J, and -N viral DNA (vDNA) fragments and *HindIII*-Q AcNPV DNA (7) were nick-translated by the method of Rigby et al. (18) and used as hybridization probes. In the case of the *EcoRI*-O probe, pAcEcoLOST (7) was restricted with *EcoRI*, the fragments were separated by agarose gel electrophoresis, and the *EcoRI*-O DNA fragment was recovered by electroelution.

DBM filters were pretreated for 12 h at 42°C in 30 ml of hybridization buffer containing 1% glycine and 10% dextran sulfate (28). Hybridization was for 18 h at 42°C in 30 ml of hybridization buffer containing 10% dextran sulfate. After hybridization, the paper was washed five times (60 min each) with continuous rocking at 42°C in 50% formamide–50 mM sodium phosphate (pH 6.5)–0.75 M NaCl–75 mM trisodium citrate–0.2% SDS (2). The DBM filters were then blotted dry, covered with SaranWrap, and exposed to X-ray film at -70°C by using Dupont Cronex Lightning Plus intensifying screens. After exposure, the probes were removed by several washes of the DBM paper with 99% formamide for 24 to 48 h with continuous rocking at 42°C . The paper was exposed to X-ray film to ensure that all of the probe was removed, pretreated as before except that the 1% glycine was omitted, and used with other probes.

RESULTS

Polypeptide synthesis in infected cells. AcNPV-infected cells (MOI, 10 PFU/cell) were pulse-labeled for 6 h with [³H]leucine at 6-h intervals from 0 to 60 h p.i. The cells were lysed at different times, and the polypeptide patterns were analyzed on 14% polyacrylamide gels (Fig. 1). A schematic diagram of the approximate time course and the relative rates of ICSP synthesis is shown in Fig. 2. The molecular weights of the high-molecular-weight polypeptides (98K, 85K, 77K, 75K, 68K, 61K, 55K, and 52K) were estimated from a 10% polyacrylamide gel (3% polyacrylamide stacking gel), which was considered to give better estimates of these polypeptides (data not shown). However, the 10% polyacrylamide gel failed to resolve any polypeptides with molecular weights below 16.5K, since all such polypeptides migrated with the gel front (data not shown). A 14% polyacrylamide gel resolved the lower-molecular-weight proteins better and was used to estimate the molecular weights of ICSPs with molecular weights ranging from 7.5K to 48K (Fig. 1 and 2).

Host cell protein synthesis gradually shut down after infection, as previously reported (6, 9, 22). The most prominent early polypeptide was ICSP 33, which was seen at 6 to 12 h p.i. This protein continued to be synthesized at a high rate until 24 h p.i., and production ceased after 30 h p.i. Based on its time course of synthesis and gel migration pattern, ICSP 33 was considered to be the 32K polypeptide described by Dobos and Cochran (9) and the 29K polypeptide of Carstens et al. (6). Other polypeptides detected during the 6- to 12-h pulse were polypeptides having molecular weights of approximately 85K, 27K, 26K, 23K, 22.5K, 19K, 18K, 13.5K, 13K, 12K, and 10.5K. On a 14% polyacrylamide gel (Fig. 1) the 85K ICSP did not resolve from the host cell 87K polypeptide; however, it migrated ahead of the host cell polypeptide (87K) when it was analyzed on 10% polyacrylamide gel (data not shown).

Labeled 10.5K polypeptide was detected between 6 and 54 h p.i., but was synthesized at its highest rate at 12 to 32 h p.i. Synthesis of the 10.5K ICSP decreased significantly after 36 h. The 10.5K polypeptide was one of the seven most abundant ICSPs detected on Coomassie blue-stained gels and was found to be soluble in 0.25 and 0.4 N sulfuric acid (data not shown). The 10.5K polypeptide was considered equivalent to an abundant basic DNA-binding core protein of baculoviruses (13, 24). This is the first report which describes the kinetics of the synthesis of this protein.

The polypeptides detected between 12 and 18 h p.i. had approximate molecular weights of 98K, 75K, 61K, 52K, 48K, 42K, 40K, 39K, 35K, 29K, 28K, 25K, 21.5K, 11.5K, 8.3K, and 7.5K. The most heavily labeled polypeptide was the 7.5K polypeptide (designated p8 by Rohel et al. [19] and 10K polypeptide by Smith et al. [23]), which was derived from an abundant class of mRNA which maps in the *EcoRI*-P region of the genome (19, 23). p8 was induced before synthesis of polyhedrin (30K), but the rates and patterns of synthesis of both polypeptides were similar between 24 and 54 h p.i. The 35K polypeptide was induced 12 to 18 h p.i. but was most actively synthesized between 24 and 48 h p.i. This polypeptide was one of the eight late polypeptides which were synthesized at high rates until 54 h p.i. (Fig. 1 and 2).

The polypeptides induced between 18 and 24 h p.i. included polyhedrin (30K), as well as polypeptides having molecular weights of approximately 77K, 68K, 55K, 43K, 20K, 16.5K, 15K, 11K, 9.6K, 9K, and 8.7K. In addition to the three polypeptides mentioned above (polyhedrin, p8, and the 35K polypeptide), the other five polypeptides synthesized at

FIG. 1. Analysis of [^3H]leucine-labeled intracellular polypeptides. *S. frugiperda* cells (8×10^5 cells per 4.5-cm^2) in 12-well plates were infected with AcNPV (MOI, 10 PFU/ml). At 6-h intervals the cells were labeled for 6 h with $30 \mu\text{Ci}$ of [^3H]leucine (59.8 Ci/mmol) per ml in 0.4 ml of leucine-free BML-TC/10 medium. At the end of each labeling period, the cells were suspended in electrophoresis sample buffer and heated in a boiling water bath for 5 min. The proteins were analyzed by electrophoresis on a 14% polyacrylamide gel ($28 \text{ by } 14.5 \text{ by } 0.15 \text{ cm}$) and then impregnated with EN 3 HANCE (New England Nuclear Corp.) and exposed to Kodak XAR-5 X-ray film at -70°C for 8 weeks. Times (hours p.i.) are indicated at the top, and the estimated molecular weights of ICSPs are indicated on the left. The positions of prestained molecular weight markers (Bethesda Research Laboratories) are indicated on the right (molecular weight $\times 10^{-3}$). UC, uninfected cells; ph, Polyhedrin.

high rates late in infection (until 54 h p.i.) had molecular weights of approximately 61K, 21.5K, 16.5K, 9K, and 8.7K. The 16.5K, 9K, and 8.7K polypeptides were synthesized at low rates between 18 and 24 h p.i., whereas between 36 and 54 h p.i. the rates of synthesis of these proteins were relatively high. The times of the maximum rates of synthesis occurred significantly later than the time of maximum extracellular virus production and coincided with an increase in the formation of occlusion bodies. Furthermore, none of these three major late-phase polypeptides could be correlated with nonoccluded virus structural polypeptides (data not shown). This suggests that these polypeptides may be involved in occlusion body formation or that they may be

constituents of the de novo synthesized membrane of the occluded virus.

To summarize, we detected 40 ICSPs in AcNPV-infected cells labeled with [^3H]leucine. The MOI, the labeling times, and the long-term pulses (6 h) were chosen to correlate with the [^3H]uridine pulses of cytoplasmic RNA (Fig. 3). Some of the polypeptides described above have not been reported previously, and some previously reported polypeptides were not detected in our ICSP analysis. In particular, we did not detect the early 48K polypeptide of Dobos and Cochran (9) (46K polypeptide of Carstens et al. [6]). This could have been due to the low content or absence of leucine compared with methionine in the 48K polypeptide, due to the higher

FIG. 2. Schematic diagram of the time course and approximate rates of ICSP synthesis derived from the data in Fig. 1. The thickness of each line indicates the intensity of the $[^3\text{H}]$ leucine-labeled ICSP according to the following arbitrary designation scheme (from thickest to thinnest line): extremely strong, very strong, strong, moderate, weak, very weak, and extremely weak (dashed line). The 6-h pulse periods are indicated at the bottom (0 to 60 h p.i.), and molecular weights (M.W.) are indicated on the left. ph, Polyhedrin.

specific activity of $[^3\text{S}]$ methionine compared with $[^3\text{H}]$ leucine, or due to a higher background of $[^3\text{H}]$ leucine-labeled host cell polypeptides after the required long exposure of the gel to the X-ray film. Furthermore, we were unable to detect any ICSP from 0 to 6 h p.i. after pulse-labeling. Similar results were reported by Dobos and Cochran (9), who used the same virus-cell system and the same MOI and detected only one early polypeptide (48K) by 6 h p.i. However, when higher MOIs (100 to 200) were used, the level of background host cell protein synthesis was reduced more rapidly and the early polypeptides were detected by 3 to 5 h p.i. (9, 16). A similar reduction in host cell protein synthesis and early appearance of ICSPs were observed when *S. frugiperda* cells were infected with first-passage virus as opposed to later-passage virus (22) or when a different cell line (*Trichoplusia ni*) was used and the virus was adsorbed by centrifugation at $1,000 \times g$ for 1 h (29). In our case, large amounts of cells were required for the preparation of cytoplasmic poly(A)⁺ RNA, which put a limit on the MOI used in these experiments.

RNA synthesis in infected cells. AcNPV-infected cells (MOI, 10 PFU/cell) were pulse-labeled for 6 h with $[^3\text{H}]$ uridine at 6-h intervals until 60 h p.i. Cytoplasmic poly(A)⁺ RNA was denatured and fractionated according to size by agarose gel electrophoresis. Figure 3 shows two exposures of the same gel; this allowed resolution of numerous RNA

species labeled 12 and 18 h p.i. and also permitted detection of minor RNA species present at other times. A total of 18 RNA species were detected by fluorography of $[^3\text{H}]$ uridine-labeled RNA (Fig. 3), but only 10 RNA species were revealed by ethidium bromide staining of the same gel (data not shown). Other RNA species not detected by labeling with $[^3\text{H}]$ uridine were identified by Northern blot hybridization (see below). Similarities were observed in the patterns of cytoplasmic poly(A)⁺ RNA and ICSP synthesis. Thus, most RNAs were detected between 12 and 24 h p.i., and synthesis of many RNAs continued until late in infection. Furthermore, the relative rates, amounts, and time courses of synthesis of the 1.4-kb RNA species (polyhedrin mRNA) and the 0.75-kb RNA species (p8 mRNA) were found to resemble those of polyhedrin and p8 polypeptides. Polyhedrin and p8 polypeptides were the two ICSPs that were synthesized at the highest rates relative to the other ICSPs (Fig. 1 and 2), and their mRNAs were also synthesized at high rates compared with the other RNA transcripts (Fig. 3). Both polyhedrin and p8 RNA transcripts were first detected at 12 to 18 h p.i., and synthesis continued at a high rate until 60 h p.i. When the same gel was stained with ethidium bromide, the 1.4- and 0.75-kb mRNAs were the most abundant RNA species detected between 24 and 60 h p.i. (data not shown). A third abundant RNA transcript, 0.6-kb RNA (which previously was shown to hybridize to the *EcoRI*-D region of the genome [19]), was first detected in the 6- to 12-h period but was synthesized at its highest rate 12 to 18 h p.i. The fourth major RNA species was the 2.8-kb RNA transcript, which was first detected between 12 and 18 h p.i. and continued to be synthesized until 48 h p.i.

FIG. 3. Analysis of cytoplasmic poly(A)⁺ RNA species from infected cells. *S. frugiperda* cells (4×10^7) were infected with AcNPV (MOI, 10 PFU/cell), and at 6-h intervals the cells were labeled for 6 h with 30 μCi of $[^3\text{H}]$ uridine (42 Ci/mmol) per ml. The cytoplasmic poly(A)⁺ RNA that was isolated at the end of each pulse-labeling period was analyzed by methyl mercury gel electrophoresis (1% agarose, 5 mM CH_3HgOH). The gel was impregnated with EN³HANCE (New England Nuclear Corp.) and exposed to Kodak XAR-5 X-ray film at -70°C . Times (hours p.i.) are indicated at the top, and the estimated sizes of RNA species (in kb) are indicated on the left. The size estimates were made by using *Escherichia coli* RNAs (23S and 16S) and globin mRNA (10S) as markers, which were localized by ethidium bromide staining of the gel after electrophoresis. (B) Shorter exposure of the same gel in order to resolve RNAs at 12 and 18 h p.i.

FIG. 4. Blot hybridization of cytoplasmic poly(A)⁺ RNAs isolated from infected cells. Cytoplasmic poly(A)⁺ RNAs isolated from infected cells at 6-h intervals from 6 to 66 h p.i. were fractionated on methyl mercury agarose gels, transferred to DBM paper, and hybridized with radioactive AcNPV DNA restriction fragments. The times of isolation of cytoplasmic poly(A)⁺ RNA (hours p.i.) are indicated at the top of each hybridization blot. Each blot is also identified by its specific hybridization probe. The estimated sizes of cytoplasmic poly(A)⁺ RNA species (in kb) are indicated on the right, and the sizes of marker RNAs are indicated on the left.

FIG. 5. Blot hybridization of cytoplasmic poly(A)⁺ RNA to *EcoRI*-B, -D, -H, and -I AcNPV DNA probes. Long exposures of hybridization blots to X-ray films are shown in order to reveal minor high-molecular-weight viral RNA species. The times (hours p.i.) of RNA isolation are indicated at the top of each blot. The estimated sizes (in kb) of the viral RNA transcripts are indicated on the right, and the sizes of RNA markers are given on the left.

Northern blot analysis of poly(A)⁺ RNA from AcNPV-infected cells. Cytoplasmic poly(A)⁺ RNA was extracted from infected cells at 6-h intervals from 6 to 66 h p.i., fractionated on denaturing methyl mercury agarose gels, transferred to DBM paper, and analyzed by hybridization with cloned restriction endonuclease fragments of AcNPV DNA (Fig. 4 and 5). The same RNA blots were used with different probes; hence, small differences in RNA mobility were judged to be significant. A schematic drawing describing the results is shown in Fig. 6. When a given probe was used, the degree of hybridization was rated as extremely

strong, very strong, strong, moderate, weak, and very weak (Fig. 6).

Two early RNAs (1.6 and 1.3 kb) detected first at 6 h p.i. were mapped in the *EcoRI*-A DNA region. A 0.75-kb RNA was also detected by the *EcoRI*-A probe. The maximum intensity of binding was 12 to 30 h p.i. An RNA transcript of similar size was shown to be mRNA for p8 polypeptide (19). However, p8 mRNA hybridized to *EcoRI*-P and *HindIII*-Q DNA fragments (19) and, as shown below, hybridized most strongly at 24 to 66 h p.i. (Fig. 4).

Seven cytoplasmic poly(A)⁺ RNAs bound to the *EcoRI*-B

FIG. 6. Schematic diagram of the blot hybridization results from Fig. 4 and 5. The thickness of each line indicates the intensity of hybridization according to the following arbitrary designation scheme (from thickest to thinnest lines): extremely strong, very strong, strong, moderate, weak, very weak, and extremely weak (dashed lines). The RNA species hybridizing to the same probe are grouped together, and size estimates (in kb) are indicated on the left. The times of RNA isolation are indicated at the bottom.

region of the genome (Fig. 4 and 5). A minor 5-kb RNA (18 to 48 h p.i.) and early 1.1-, 1.5-, and 1.75-kb RNAs (6 h p.i.) were detected only after prolonged exposure of the blot (Fig. 5). The RNA species of interest in this region of the genome were a major 1.5-kb RNA transcript (6 to 66 h p.i.) and a late 2.6-kb RNA detected between 24 and 66 h p.i. Three RNA transcripts (0.95, 1.3, and 2.6 kb) were previously mapped in this region of the genome by a Northern blot analysis of cytoplasmic poly(A)⁺ RNA isolated at 48 h p.i. (23). The translation product of the late 2.6-kb RNA is unknown, but since the transcript accumulated very late in the infection cycle, after the onset of occlusion body formation, the protein could either be a constituent of the de novo synthesized membrane of the occluded virus or be involved in occlusion body morphogenesis. The early 1.75-kb RNA (6 h p.i.) was detected only after prolonged exposure of the blot, and its possible presence at other times after infection was obscured by other transcripts (Fig. 4 and 5).

The strongest hybridization on the Northern blot probed with *EcoRI*-C DNA was hybridization to 1.4-kb RNA, whereas the *EcoRI*-D and *EcoRI*-E blots showed strongest hybridization to 0.6- and 2.8-kb RNAs, respectively. A

minor 4.9-kb RNA (12 to 48 h p.i.) and an early 1.5-kb RNA (6 h p.i.) were detected on an *EcoRI*-D blot only after prolonged exposure (Fig. 5). Minor amounts of an abundant 3.5-kb RNA (*EcoRI*-D) were also detected at 6 h p.i., but only after prolonged overexposure, which totally obscured all RNA transcripts observed at later times (data not shown). Two moderately abundant transcripts, 4.2-kb RNA (12 to 48 h p.i.) and 3.4-kb RNA (24 to 66 h p.i.), hybridized to the *EcoRI*-H region. The 3.4-kb RNA was a very late transcript (24 to 66 h p.i.), and its protein may also have a role in occlusion body assembly or be a constituent of the de novo synthesized membrane of the occluded virus. The three RNAs (2.3, 2.0, and 1.5 kb) which hybridized to *EcoRI*-H vDNA had the same estimated sizes as three species which bound to *EcoRI*-E vDNA, and they probably overlapped the *EcoRI*-E-*EcoRI*-H junction. A minor 4.9-kb RNA (12 to 48 h p.i.) hybridized to *EcoRI*-H DNA and was detected after prolonged exposure of the blot to X-ray film (Fig. 5).

Six RNAs hybridized to *EcoRI*-I vDNA. Short film exposures allowed resolution of four RNAs (Fig. 4), but the other two species (5.2 and 1.7 kb) required a longer exposure (Fig. 5). The 1.7-kb RNA could only be resolved at 6 h p.i., and its

FIG. 7. Transcription map for AcNPV: linearized physical map of AcNPV HR3 DNA (7) with the appropriate locations of viral RNA transcripts, which are designated by their estimated sizes (in kb). Previously published translational maps for AcNPV are shown at the top. The heavy black areas on the physical map of AcNPV DNA indicate the regions of homology (8). Kbp, Kilobase pairs.

possible presence at later times was obscured by other transcripts. The major RNA transcript within the *EcoRI*-I region was polyhedrin mRNA (1.4 kb) (19, 23), which was detected by the probe from 12 to 66 h p.i. (Fig. 4). Other transcripts from this region were 2.5-, 3.2-, and 4.2-kb RNAs. The 2.5-kb RNA was observed only 12 to 30 h p.i. The polyhedrin message and 3.6- and 5.4-kb RNAs were also reported by Smith et al. (23), who used a *HindIII*-V vDNA probe. It is likely that the 3.2- and 4.2-kb transcripts overlapped the *EcoRI*-I-*EcoRI*-B junction since RNAs of these sizes hybridized to both adjacent *EcoR* fragments.

The principal RNA which bound to *EcoRI*-J DNA was 1.2 kb long. This RNA showed strong hybridization between 12 and 48 h p.i.; slight binding was observed at 6 h p.i. and also at 60 and 66 h p.i. Four RNAs hybridized to the *EcoRI*-N DNA fragment, whereas one RNA transcript hybridized to the *EcoRI*-O DNA fragment. The major RNA transcript in the *HindIII*-Q region of the genome was 0.75-kb RNA, which was previously shown to be the mRNA for an abundant p8 polypeptide (19). Figure 4 shows extremely strong hybridization of p8 mRNA to the *HindIII*-Q DNA fragment between 12 and 66 h p.i. Other RNAs hybridizing to this region of the genome were 2.7-kb RNA (18 to 66 h p.i.), 3.1-kb RNA (18 to 48 h p.i.), 1.6-kb RNA (12 to 30 h p.i.), the early 1.3-kb RNA (6 to 30 h p.i.), and the early 1.0-kb RNA seen at 12 h p.i. (Fig. 4) but also detected at 6 h p.i. after prolonged exposure (data not shown). The abundant 0.75-kb transcript (12 to 66 h p.i.) made detection of the 1.0-kb RNA difficult between 18 and 66 h p.i. (Fig. 4).

DISCUSSION

Information on transcription and translation mapping is needed in order to understand gene organization and expression of the AcNPV genome. Several investigators have used hybridization selection to map virus-encoded polypeptides (1, 11, 22). However, very little is known about the viral transcripts.

The results shown in Fig. 1 and 2 confirm that polypeptide synthesis is under temporal control. Figure 3 shows that polyhedrin and p8 mRNAs were synthesized at high rates from 12 to 66 h p.i. and that the previously observed abundance of these two transcripts at late times after infection (19) was due to continuous and abundant transcription of the genes. Furthermore, our results suggest that both the abundance and the temporal controls of polyhedrin and p8 polypeptide synthesis are at the level of transcription.

We also extracted poly(A)⁺ RNA at intervals in the replication cycle and analyzed the size classes, relative amounts, and gene locations of these RNA transcripts. A transcription map was generated from Northern blot hybridization data by using cytoplasmic poly(A)⁺ RNA analyzed at 6-h intervals in infected cells.

Our data confirm that there are many transcripts of interest which are not yet linked to translation products. Examples are "early" 1.8- and 1.3-kb RNAs which hybridized to *EcoRI*-A vDNA; "late" 2.7-kb and predominant "middle period" 1.6-kb RNA transcripts (*HindIII*-Q); an abundant middle period 0.6-kb transcript (*EcoRI*-D); a 1.4-kb RNA transcript (*EcoRI*-C); major middle period (4.2-kb) and major "very late phase" (3.4-kb) RNA transcripts (*EcoRI*-H); the middle period 2.5-kb RNA (*EcoRI*-I); major RNA transcripts (2.8 and 1.2 kb) binding to *EcoRI*-E and -J DNA fragments, respectively; a 1.3-kb RNA (*EcoRI*-O); major 1.5-kb and minor middle period 4.2- and 3.2-kb RNAs; and a "very late period" 2.4-kb RNA transcript which hybridized to the *EcoRI*-B region of the genome.

When the transcription data are correlated with previously published translational maps (Fig. 7), some similarities and differences are noted. We found six RNAs (0.6, 1.2, 1.9, 2.1, 3.5, and 4.9 kb) which hybridized to the *EcoRI*-D DNA fragment at 24 h p.i., and Esche et al. (11) mapped five viral polypeptides (21K, 25K, 34K, 49K, and 50K) in this region of the genome. Similarly, six RNAs (1.1, 1.5, 2.6, 3.2, 4.2, and 5.0 kb) hybridized to the *EcoRI*-B region at 24 h p.i., and five polypeptides (16K, 25K, 36K, 40K, and 52K) also mapped in this region of the genome (11). Further examples of correlation between viral transcripts and polypeptide synthesis throughout the genome are given in Fig. 7, but in some regions little correlation was noted (for example, in loci containing the *EcoRI*-E, -H, and -I and *HindIII*-Q vDNA fragments). Knowledge of the size and abundance of RNA transcripts should prove useful in further translation mapping and in the isolation of virus mRNAs.

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