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In Vitro Translation of *Autographa californica* Nuclear Polyhedrosis Virus Early and Late mRNAs

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A preliminary translational map of the *Autographa californica* genome was constructed. Eighteen viral DNA restriction fragments were either purified from agarose gels or obtained from pBR322 recombinant DNA plasmids to locate specific gene products. The DNAs were immobilized on nitrocellulose filters and used to select viral mRNAs isolated from RNA obtained from the cytoplasm of infected *Spodoptera frugiperda* cells at 21 h postinfection. The fragment-specific mRNAs were translated in vitro in the presence of L-[^{3}H]leucine by using a rabbit reticulocyte lysate system and analyzed on sodium dodecyl sulfate-polyacrylamide gels. The approximate locations of 19 *A. californica* nuclear polyhedrosis virus (AcMNPV) gene products were mapped. The genes for mRNAs present late in viral infection were mapped to DNA fragments that represent nearly the entire genome. The molecular weights of many of these proteins were similar to those present in purified AcMNPV extracellular virus and to proteins being made in infected cells at 18 to 21 h postinfection. Cytoplasmic RNA was isolated at 4 h postinfection from infected cells, a time early in the viral infection cycle, and hybridized to AcMNPV DNA immobilized on nitrocellulose filters. AcMNPV-specific early RNA was translated in vitro into at least six polypeptides, the most abundant having a molecular weight of 39,000. Viral polypeptides were detected in cells pulse-labeled with L-[^{3}H]leucine at 3 to 6 h postinfection, with molecular weights similar to those of polypeptides made in vitro from early AcMNPV mRNA.

The *Autographa californica* nuclear polyhedrosis virus (AcMNPV) genome is about 85 × 10^6 daltons and is large enough to code for 75 or more proteins. Several reports, using pulse-labeling of AcMNPV-infected cells with radioactive amino acids, have demonstrated at least two temporal classes of virus-induced proteins: early polypeptides made before DNA synthesis (about 6 h postinfection [p.i.]) and late polypeptides made after DNA synthesis (1, 3, 5, 8, 16, 17). Hybridization and translation analyses of mRNA obtained from AcMNPV-infected cells have shown that by 21 h p.i., late in the viral infection cycle, essentially all of the cytoplasmic mRNAs are virus specific (16). It was also demonstrated that RNA sequences complementary to *Eco*RI restriction fragments I and J are translated in vitro into polypeptides with molecular weights of 33,000 (33K) and 39K, respectively. The 33K protein was identified as AcMNPV polyhedrin, the major protein component of AcMNPV occlusions.

To study the organization and expression of the AcMNPV genome further, it is necessary to know the location and temporal expression of individual genes. One approach used to map the location of viral genes is to hybridize infected cell RNA to DNA fragments immobilized on filters, selectively elute the restriction fragment-specific RNA, translate the mRNA in vitro, and characterize the radiolabeled translation products by polyacrylamide gel electrophoresis. In the present study we show that AcMNPV RNA isolated from cells at early and late stages of infection can be translated in vitro into a large number of virus-specific proteins. Many of the in vitro translation products made from late viral mRNA had the same molecular weights as those of the virion structural proteins. The approximate location of numerous AcMNPV genes was determined. Sequences complementary to polyhedrin mRNA were mapped onto *Hin*dIII fragment V. Virus-specific early RNA was purified from infected cells at 4 h p.i. and was translated into at least six polypeptides. The possible location of the AcMNPV genes expressed early in the infection cycle is discussed.

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MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* IPLB-SF21 (Sf) cells were grown at 27°C in Grace insect tissue culture medium (KC Biologicals) containing 10% calf serum and no antibiotics. The cells were maintained in spinner cultures, and 4×10^7 log-phase cells were seeded in plastic Corning tissue culture flasks (150 cm^2) before inoculation with virus. A plaque-purified isolate of AcMNPV, E2 (11), was used to infect cells at a multiplicity of infection (MOI) of 20 PFU/cell. The following procedure was used to produce high-titer first-passage virus used in these experiments. *Trichoplusia ni* larvae were infected with AcMNPV E2 virus per os. At 4 to 5 days p.i. the hemolymphs of diseased larvae were collected into an equal volume of 10 mM Tris (pH 6.8)–2 mM dithiothreitol–150 mM NaCl, diluted with Grace medium, and used to infect Sf cells at an MOI of 0.1 PFU/cell. At 48 h p.i., medium containing extracellular virus (ECV; first-passage virus) was separated from infected cells by differential centrifugation. The virus-containing medium had titers of 2×10^8 to 3×10^8 PFU/ml. No significant reduction in titer was observed when the inoculum was stored at 4°C for 1 year.

Radiolabeled proteins and gel electrophoresis. To label cell proteins, we infected Sf cells at an MOI of 20 PFU/cell. The cells were washed at various times after infection in defined medium (11) lacking L-leucine and pulse-labeled for 3 h in defined medium containing 100 μCi of L-[4,5-^{3}H]leucine (46 Ci/mmol; ICN) per ml. The labeling medium was removed, and the cells were washed twice with ice-cold phosphate-buffered saline and boiled for 3 min in sodium dodecyl sulfate protein disruption buffer (125 mM Tris-hydrochloride [pH 6.8], 2% sodium dodecyl sulfate, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromophenol blue). Proteins were analyzed on 10 or 12% polyacrylamide slab gels as described by Laemmli (6); electrophoresis was at 9 W (constant power) for 3 h. Fluorography was performed as described by Laskey and Mills (7). The gels were exposed to Kodak X-Omat RP film at −80°C. Protein molecular-weight standards (Amersham Corp.) were ^{14}C-methylated phosphorylase (molecular weight, 93K), bovine serum albumin (69K), ovalbumin (46K), and lysozyme (14K).

Purification of ^{3}H-labeled ECV. Sf cells (4×10^7 cells per 150-mm^2 Corning cell culture flask) were infected at an MOI of 10 with AcMNPV first-passage virus. At 4 h p.i. the cells were washed twice with defined medium, and then 20 ml of defined medium with 1 mCi of L-[4,5-^{3}H]leucine was added to each flask. At 48 h p.i. the ^{3}H-labeled ECV was purified by sucrose density gradient centrifugation as described previously (11).

Isolation of RNA from infected cells. Infected cells were harvested at 4 or 21 h p.i. and washed with ice-cold phosphate-buffered saline. The cells were lysed in 30 mM Tris-hydrochloride (pH 7.5)–10 mM magnesium acetate–1% Nonidet P-40 for 10 min on ice and were intermittently blended in a Vortex mixer. The nuclei were pelleted by centrifugation at $1,500 \times g$ for 5 min, and the supernatant (cytoplasmic fraction) was poured into sterile polypropylene centrifuge tubes. A 0.1 volume of 2.0 M potassium acetate (pH 5.0)–4% sodium dodecyl sulfate–0.30 M EDTA was added, and the RNA was extracted twice with phenol (saturated with 10 mM Tris-hydrochloride [pH 7.5]–10 mM EDTA)-chloroform-isoamyl alcohol (24:24:1) and twice with chloroform-isoamyl alcohol (24:1). Two volumes of ethanol were added to the aqueous phase, and the RNA was precipitated at −80°C for 15 min. The RNA precipitate was collected by centrifugation at $3,000 \times g$ for 30 min, washed twice with 95% ethanol, lyophilized, suspended in sterile distilled water, and stored at −80°C. Polyadenylic acid-containing RNA [poly(A)$^+$ RNA] was isolated by oligodeoxythymidylic acid-cellulose chromatography as described previously (16).

Purification of DNA restriction fragments. AcMNPV DNA was isolated from purified virus and cleaved with restriction enzymes as described previously (12). Individual restriction fragments were isolated from agarose gels after electrophoresis, using the electrophoretic extraction method described by Smith and Summers (12). Three of the DNA restriction fragments used in this study (*Hin*dIII fragments F, V, and T) had been cloned in pBR322 plasmids (Smith et al., manuscript in preparation). Recombinant DNA plasmids were amplified and purified as described by Holmes and Quigley (4). All of the cloning experiments were conducted in accordance with the current National Institutes of Health guidelines.

Hybridization selection and in vitro translation of AcMNPV RNA. Cytoplasmic RNA was hybridized to AcMNPV DNA, which had been immobilized on nitrocellulose filters, as described by McGrogan et al. (9), with minor modifications (16). AcMNPV DNA fragments, isolated from 100 μg of total DNA restriction digests, were bound to individual filters (1-cm diameter) and hybridized to 1 mg of cytoplasmic RNA. Translation of RNA was performed in an mRNA-dependent rabbit reticulocyte lysate system (Bethesda Research Laboratories, Inc.) as described previously (16). In vitro-synthesized proteins were labeled with L-[4,5-^{3}H]leucine (55 Ci/mmol; ICN).

RESULTS

Infected cell-specific proteins. The induction of AcMNPV-infected cell-specific proteins (ICSPs) and the reduction of host cell protein synthesis have recently been shown to be affected by the MOI (8). At a high MOI, ICSPs were detected earlier and host cell protein synthesis was reduced more rapidly than at a low MOI. We also observed that background host cell protein synthesis was lower in Sf cells infected with first-passage virus than in cells infected with later-passage virus (data not shown). Therefore, in the present report, Sf cells were infected with first-passage virus at an MOI of 20. These conditions resulted in the appearance of many polyhedra in most cells between 18 and 21 h p.i., indicating an apparently synchronous infection.

A comparison of proteins in infected Sf cells labeled with L-[^{3}H]leucine at early (3 to 6 h p.i.) and late (18 to 21 and 48 to 51 h p.i.) times is shown in Fig. 1. Host cell protein synthesis (lane a) was slightly reduced by 3 to 6 h p.i. (lane b), and was minimal at 18 to 21 h p.i. (lane c). At 48

to 51 h p.i. (lane d) most ICSPs were no longer synthesized, except for two major late proteins with molecular weights of 33K and 10K. Since the molecular weights of the low-molecular-weight proteins are not accurately measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Laemmli (6), we consider these to be only estimates. At 18 to 21 h p.i. at least 35 ICSPs were synthesized, and several ICSPs were detected early at 3 to 6 h p.i. About 25 ICSPs that were produced between 18 and 21 h p.i. had electrophoretic mobilities similar to those of polypeptides present in purified ECV (Fig. 2, lane c). To better resolve the low-molecular-weight proteins, pulse-labeled cell proteins (18 to 21 h p.i.) and purified ECV were electrophoresed in a 12% polyacrylamide gel (Fig. 2, lanes d and e, respectively). For each of the virus structural proteins (indicated by their molecular weights in Fig. 2, lanes c and e), there was a polypeptide of the same molecular weight being made in infected cells between 18 and 21 h p.i. (Fig. 2, lanes a and d). The other ICSPs, which may be either occluded virus structural proteins or nonstructural virus proteins, are listed in Fig. 2, lane a.

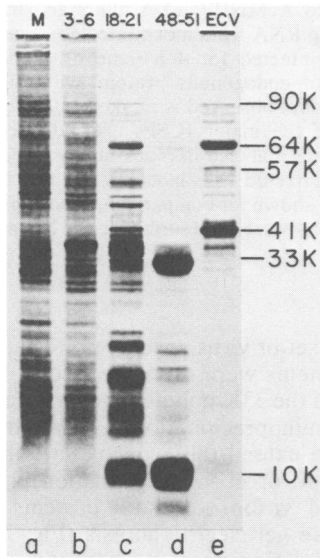

FIG. 1. Comparison of pulse-labeled infected cell proteins and purified AcMNPV ECV proteins. Autoradiogram of mock-infected (lane a) and AcMNPV-infected (lanes b through d) *S. frugiperda* cells and ECV (lane e). Cells were labeled for 3 h with L-[^{3}H]leucine at 3 to 6 h p.i. (lane b), 18 to 21 h p.i. (lane c), or 48 to 51 h p.i. (lane d). ECV was obtained from infected cell cultures continuously labeled from 4 to 48 h p.i. with L-[^{3}H]leucine. The molecular weights of the polypeptides are indicated (× 10^3). The proteins were analyzed on a 10% polyacrylamide gel, prepared for fluorography, and exposed for 4 days at −80°C.

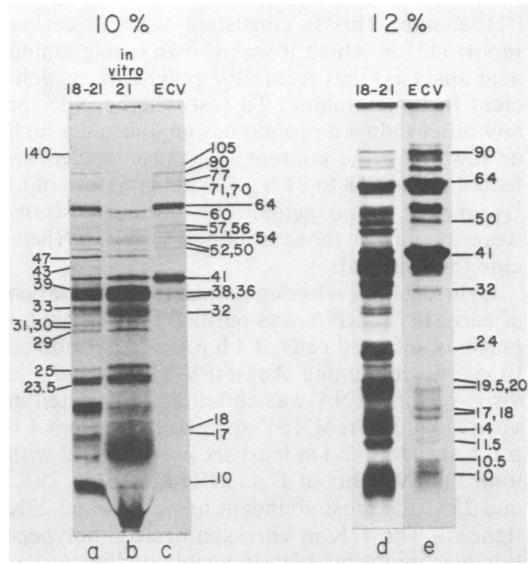

FIG. 2. A comparison of in vivo- and in vitro-synthesized infected cell proteins. AcMNPV-infected *S. frugiperda* cells, pulse-labeled with L-[^{3}H]leucine at 18 to 21 h p.i., and L-[^{3}H]leucine-labeled ECV were electrophoresed in 10% polyacrylamide (lanes a and c, respectively) and 12% polyacrylamide gels (lanes d and e, respectively). Poly(A)$^+$ RNA was isolated from AcMNPV-infected cells at 21 h p.i., and translated in vitro in the presence of L-[^{3}H]leucine (lane b). Molecular weights of the ECV polypeptides are indicated (× 10^3) adjacent to lanes c and d. Molecular weights of additional ICSPs, not observed in purified ECV, are indicated (× 10^3) adjacent to lane a. Fluorography was as described in the legend to Fig. 1.

The 10K polypeptide was the most abundant labeled ICSP being synthesized between 18 and 21 h p.i. (Fig. 1, lane d); but it was not detected early in infection (3 to 6 h). The 10K polypeptide was neither a major component of AcMNPV ECV (Fig. 1, lanes c and e) nor a major structural protein in AcMNPV occluded virions purified from polyhedra (not shown). AcMNPV polyhedrin (33K) was not being made at a high level in infected cells at 18 to 21 h p.i. However, by 48 to 51 h p.i., polyhedrin and the 10K polypeptide were the major translation products (Fig. 1, lane d).

When infected cells were pulse-labeled with L-[^{35}S]methionine instead of L-[^{3}H]leucine, no 10K polypeptide could be detected up to 48 h p.i. (not shown). Therefore, it is likely that the 10K polypeptide is deficient in the amino acid L-methionine and thus was not detected in previous investigations in which L-[^{35}S]methionine was used as a radioactive tracer (1, 3, 5, 8, 17). The polyhedrin protein was also less efficiently labeled with L-[^{35}S]methionine than with L-

[^{3}H]leucine. This is consistent with an earlier report (13) in which it was shown (using amino acid analysis) that AcMNPV polyhedrin is deficient in L-methionine. To test whether 10K or any other induced protein has an unusually high or low L-leucine content, we pulse-labeled infected cells at 18 to 21 h p.i. with a mixture of L-^{3}H-labeled amino acids. The protein patterns were similar to those obtained with L-[^{3}H]leucine (not shown).

Hybridization selection and in vitro translation of early RNA. RNA was purified from the cytoplasm of infected cells at 4 h p.i. and hybridized to filters containing AcMNPV DNA, and the virus-specific RNA was eluted and translated in vitro (Fig. 3). AcMNPV-specific RNA from 4 h p.i. translated into at least six polypeptides with molecular weights of 47K, 39K, 32K, 31K, 29K, and 25K, the most abundant being 39K and 29K (lane d). The 47K in vitro-synthesized polypeptide was minor but clearly visible on the autoradiogram. There were also some low-molecular-weight proteins synthesized from virus-specific RNA which could be seen in lane d when the autoradiogram was exposed longer (not shown). An ICSP of 39K was the major virus-induced polypeptide observed in cells pulsed-labeled from 3 to 6 h p.i. (lane g). ICSPs of 47K, 32K, 31K, and 29K were also detected in these cells. Although it is possible that the in vitro-translated 25K protein was due to premature termination and not to an authentic early protein, we have recently used cloned DNA restriction fragments and have selected for an early mRNA that translated into a 25K polypeptide (Smith et al., in preparation). A control of the same amount of mock-infected Sf cell RNA was hybridized to similar AcMNPV DNA filters. No detectable polypeptides were synthesized from mock-infected-cell RNA selected against AcMNPV DNA filters (lane b).

Translation of poly(A)$^+$ RNA from cells isolated at 4 h p.i. (Fig. 3, lane e) showed numerous polypeptides with electrophoretic mobilities similar to those of polypeptides from poly(A)$^+$ RNA from uninfected cells (lane c), with the exception of a few virus-specific polypeptides (lane d). For example, 39K, 29K, and 25K polypeptides were easily detected among the translation products from RNA isolated at 4 h p.i. (compare lanes e and c). This indicated that poly(A)$^+$ RNA present at 4 h p.i. was predominantly of host cell origin, in contrast to poly(A)$^+$ RNA isolated at 21 h p.i. (lane f), which has been shown to be virus specific (16).

In vitro translation of 21-h RNA. In a previous study (16) we demonstrated that poly(A)$^+$ RNA and AcMNPV-specific RNA obtained by hybridization selection from cytoplasmic RNA isolated at 21 h p.i. could be translated into an identical set of virus-specific proteins. A few of these proteins were the same size as ECV proteins, and the 33K polypeptide was identified by using immunoprecipitation as polyhedrin. In this report we extend this information further by a comparison of in vitro translation products to ICSP and virion structural proteins by polyacrylamide gel electrophoresis (Fig. 2). The in vitro translation products between about 16K and 64K daltons were similar both in size and in relative abundance to those made in infected cells (Fig. 2, lanes a and b). Only two polypeptides (with molecular weights of 20K and 30K) were present in infected cells but were not detected among the in vitro translation products. A minor polypeptide of 31K was present in in vitro-synthesized proteins, but no corresponding polypeptide was found in infected cells. Five virion proteins (from 70K to 105K) were synthesized at a lower level than that observed in

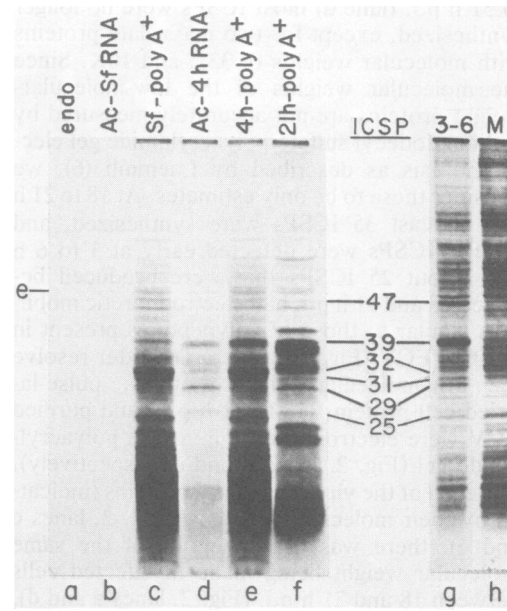

FIG. 3. In vitro translation of AcMNPV early RNA. Poly(A)$^+$ RNA was translated in vitro after isolation from mock-infected cells (lane c), cells infected for 4 h (lane e), and cells infected for 21 h (lane f). AcMNPV-specific RNA was isolated by hybridization selection to AcMNPV DNA filters and translated in vitro, using RNA from mock-infected Sf cells (lane b) and cells infected for 4 h (lane d). Lane a shows products of endogenous protein synthesis, and the major product observed is marked (e). The molecular weights of the major ICSPs are indicated ($\times 10^3$). Proteins pulse-labeled in mock-infected cells (M; lane h) and in infected cells labeled for 3 to 6 h p.i. (3–6; lane g) are shown for comparison. Electrophoresis and fluorography were as described in the legend to Fig. 1.

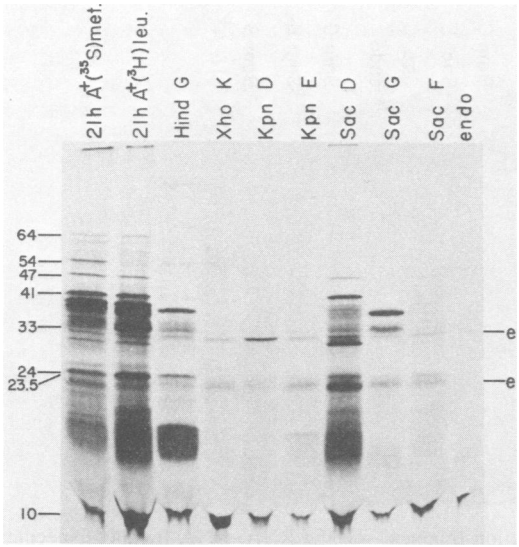

FIG. 4. In vitro translation of AcMNPV DNA restriction fragment-selected RNA. RNAs were selected by hybridization to purified DNA fragments, eluted, and translated in vitro in the presence of L-[^{3}H]leucine. Poly(A)$^+$ RNA isolated from cells at 21 h p.i. was translated in vitro and labeled with either L-[^{35}S]methionine or L-[^{3}H]leucine, and the translation products were electrophoresed in the first and second slots, respectively. The molecular weights of several in vitro translation products are indicated ($\times 10^3$). Polypeptides observed in an endogenous protein synthesis reaction are marked (e). Proteins were electrophoresed in 10% polyacrylamide gels, and fluorography was for 12 days at −80°C.

infected cells (Fig. 2). We have not detected in vitro translation products larger than 105K. Whether high-molecular-weight mRNAs were selectively degraded during isolation or whether they were not as efficiently translated in vitro is not known.

The presence of a large quantity of unlabeled globin and serum albumin in the reticulocyte lysate used for in vitro translation interfered with the mobilities of 10K and 68K polypeptides, respectively. RNA isolated 21 h p.i. and translated in vitro produced a large amount of labeled protein that migrated in the gel just below rabbit globin (Fig. 2, lane b). In a control in which no exogenous RNA was added to the in vitro translation system there was only a minor amount of labeled protein in this same region (Fig. 4). Therefore, the mRNA for the 10K protein was most likely present in RNA isolated at 21 h p.i. and translated in vitro. We also observed a slight increase in the electrophoretic mobility of the 64K protein, probably due to the interference from rabbit serum albumin. It is also possible that the slight difference in molecular weights was due to the fact that in infected cells this protein is modified, whereas when translated in vitro it is not.

In vitro translation of restriction fragment-specific AcMNPV RNA. AcMNPV DNA restriction fragments were isolated from preparative agarose gels, and the identities and purities of the fragments were checked by analytical agarose gels as detailed previously (16). Restriction fragments that could be easily separated from other fragments were chosen to minimize contamination. The 18 DNA fragments used (see Fig. 6 [in black]) are representative of nearly all of the AcMNPV genomes. *Hin*dIII fragments F, V, and T were obtained from purified recombinant plasmid DNAs. To avoid competition with the same viral DNA sequences for RNAs during hybridization, each hybridization mixture contained filters with DNA fragments having nonoverlapping sequences. For example, filters containing *Hin*dIII fragments G and F were not included in the same hybridization mixture with filters containing *Bam*HI fragment B.

The in vitro translation products obtained from restriction fragment-selected RNAs are shown in Fig. 4 and 5. We have shown in a previous study (16) that an mRNA for a 39K polypeptide was selected from *Eco*RI-J and that AcMNPV polyhedrin mRNA is selected by *Eco*RI fragment I. Four DNA fragments were chosen, representing the sequences within *Eco*RI fragment I: *Hin*dIII fragments F, V, and T and *Bam*HI fragment C. At least one of these fragments would be expected to have sequences complementary to polyhedrin mRNA. A 33K polypeptide was translated only from RNA selected by *Hin*dIII fragment V. Thus, sequences complementary to polyhedrin mRNA included *Hin*dIII fragment V. The approximate location of other AcMNPV genes was determined by comparing the molecular weights of in vitro translation products of RNAs isolated from DNA fragment filters. For example, a 41K polypeptide was made from *Sac*I fragment D-specific RNA (Fig. 4), and a 41K polypeptide was also made from *Sma*I fragment C-specific RNA (Fig. 5). Therefore, sequences that at least in part specify for the 41K were mapped to the region of the genome that is common to both *Sac*I fragment D and *Sma*I fragment C (Fig. 6). The AcMNPV translation products that were mapped in this manner are listed in Table 1, and the approximate location of the genes that specify for these proteins is shown in Fig. 6. Most of the viral genes were mapped from an analysis of in vitro translation products made from viral RNAs selected from overlapping DNA restriction fragments as described above. Because no overlapping DNA fragments for translation products of 23.5K, 25K, 32K, 36K, and 38K

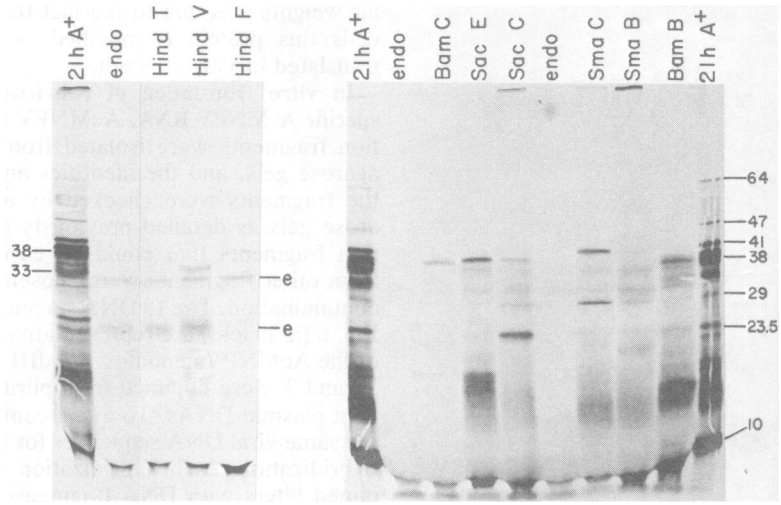

FIG. 5. In vitro translation of AcMNPV DNA restriction fragment-specific RNA. DNA fragment-specific RNAs were translated in vitro and electrophoresed as described in the legend to Fig. 4. Fluorography was for 14 days at −80°C.

polypeptides were included in these experiments, their locations have not been confirmed. The mRNAs for 23.5K and 38K were selected for by adjacent restriction fragments: *Sac*I-C and -F and *Hin*dIII-G and -F, respectively. This suggests that the gene sequences for 23.5K and 38K polypeptides are on adjacent restriction fragments (Fig. 6).

A significant increase in the amount of radiolabeled protein which migrates just in front of the approximately 10K globin band was observed with RNA selected from the overlapping *Xho*I fragment K and *Bam*HI fragment B. This suggests that the gene for a 10K polypeptide includes sequences in *Xho*I fragment K. Hybridization analysis of ^{32}P-labeled cDNA synthesized from RNA isolated at 48 h p.i. and northern gel analysis have also located a major late transcript from this region of the genome (Smith et al., in preparation). However, an increased amount of labeled protein migrating in front of rabbit globin was also observed with RNA selected from three other regions of the genome, i.e., *Sac*I fragments D, E, and F. Whether these are authentic low-molecular-weight viral translation products, artifacts caused by degradation of RNA during hybridization, or products of premature termination of in vitro translation is not known. Additional minor in vitro translation products were observed with several restriction fragment-selected RNAs. We do not know the origin of these proteins.

To detect any proteins that are deficient in either L-leucine or L-methionine, the poly(A)$^+$ RNA isolated at 21 h p.i. was translated in vitro and labeled with L-[^{3}H]leucine or L-[^{35}S]methionine as a radioactive tracer, and the products were compared by gel electrophoresis (Fig. 4). The 10K polypeptide was not as efficiently labeled with L-methionine as with L-leucine, which was similar to the results seen in pulse-labeled cells. Polyhedrin (33K) was more efficiently labeled with L-leucine, most likely due to the fact that AcMNPV polyhedrin is deficient in

TABLE 1. Location of AcMNPV in vitro translation products

In vitro translation product (mol wt)	mRNA derived from DNA fragments[a]:
64K	*Sma*I-B and *Kpn*I-D
57K	*Sma*I-B and *Kpn*I-D
54K	*Bam*HI-B and *Hin*dIII-G, but not *Xho*I-K
52K	*Sma*I-B and *Kpn*I-D
47K	*Sma*I-C and *Sac*I-D
41K	*Sma*I-C and *Sac*I-D
39K	*Sac*I-E, *Eco*RI-J, and possibly *Sac*I-C
38K	*Bam*HI-B and *Hin*dIII-G and F
38K	*Bam*HI-C, but not *Eco*RI-I
36K	*Sac*I-G or H
33K	*Eco*RI-I and *Hin*dIII-V
32K	*Sac*I-G or H
31K	*Sma*I-B and *Kpn*I-D
29K	*Sma*I-C and *Sac*I-D
25K	*Sac*I-E, but not *Eco*RI-J
24K	*Bam*HI-B and *Hin*dIII-G, but not *Xho*I-K
24K	*Sac*I-D, but not *Sma*I-C
23.5K	*Sac*I-C and *Sac*I-F
10K	*Bam*HI-B and *Xho*I-K, but not *Hin*dIII-G

[a] *Eco*RI-I- and *Eco*RI-J-specific mRNA in vitro translation products were as reported previously (16).

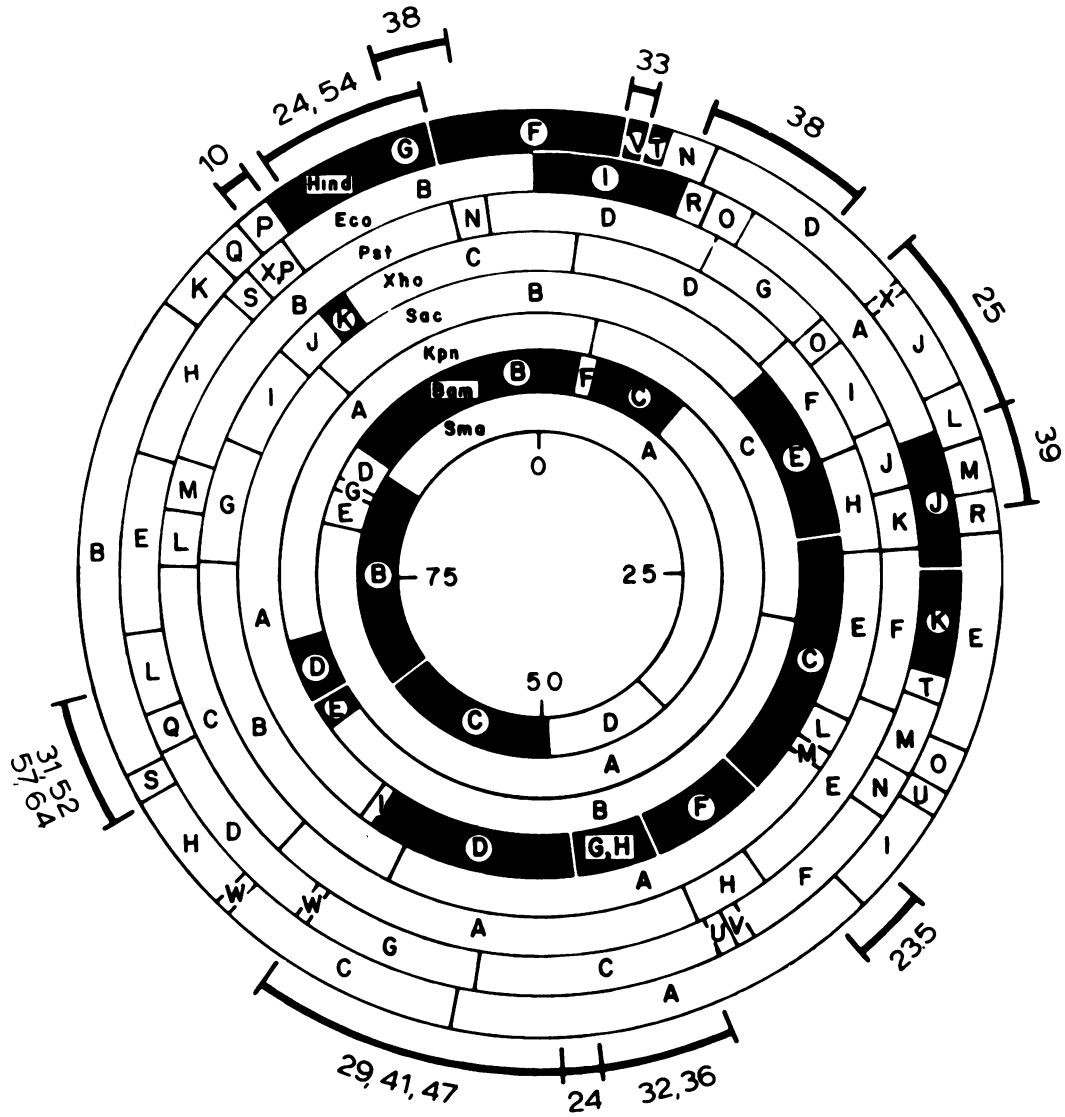

FIG. 6. Map of AcMNPV in vitro translation products. The restriction map is oriented with 0% between *Eco*RI-B and I and the location of restriction sites is as described by Vlak and Smith (15a). The black restriction fragments were those used for hybridization selection of AcMNPV mRNA. The bars around the outside of the map define the location of AcMNPV translation products that have been mapped as described in the text. The identity of a specific gene product is indicated by its molecular weight $\times 10^3$.

L-methionine (13). On the other hand, a 54K polypeptide incorporated more L-methionine than L-leucine (Fig. 4). Since L-[^{3}H]leucine was used in our translation experiment, we were not able to locate the 54K protein gene on the genome.

DISCUSSION

The molecular weights and relative abundance of virus-induced early and late polypeptides in AcMNPV-infected cells were similar to those reported in previous studies (1, 3, 5, 8, 16, 17). One exception was a major AcMNPV-induced polypeptide of about 10K. When infected cells were pulse-labeled with L-[^{3}H]leucine at 18 to 21 h p.i., 10K was the most abundant labeled polypeptide. By 48 h p.i. nearly all of the protein being made in infected cells was either 10K or polyhedrin (33K), and both were being made at approximately the same rates. In addition, both of these viral proteins accumulate in infected *S. frugiperda* cells (Smith et al., in preparation).

The function of 10K protein is not known, but it is a major viral gene product that is a very minor component of AcMNPV virions, and its synthesis cannot be detected until late in the infection cycle. At 21 h p.i. the 10K protein is being made at high levels, whereas polyhedrin is not; at 48 h p.i., both proteins are synthesized in abundance. This suggests that the expression of 10K and polyhedrin genes is being differentially regulated at 21 h p.i. Since 10K and 33K were mapped about 17 kilobases apart, it is possible that they are under the control of separate promoters. However, RNA splicing of leader sequences is known to occur over this distance, e.g., in adenovirus-2 late mRNAs (10). There might also be controls over the expression of 10K and polyhedrin which are similar since very late in the infection cycle both gene products are made at high levels.

Purified AcMNPV ECV is composed of at least 25 polypeptides of which the 41K and 64K polypeptides are two major components (11, 15) that are labeled with L-[^{3}H]leucine. A polypeptide with a molecular weight of about 17.5 K is also a major AcMNPV virus structural protein found both in AcMNPV ECV and in virions purified from occlusions (11). This protein is not labeled with either L-[^{35}S]methionine (11) or, as shown in the present report, with L-[^{3}H]leucine. The 41K polypeptide is a major viral capsid protein (14), and the 64K polypeptide is a major ECV protein but a minor component of occluded viruses (11, 15). The 64K polypeptide is phosphorylated and occurs predominantly in the cytoplasm of infected cells (8). Polypeptides of 41K and 64K were among the proteins translated in vitro from RNA isolated at 21 h p.i. In a previous study (16) it was shown that AcMNPV polyhedrin is present in infected Sf cells at 21 h p.i. and that a polypeptide of the same size can be translated in vitro from RNA isolated at 21 h p.i. and immunoprecipitated with polyhedrin-specific antiserum. In that study and in the present report we observed a good correlation between the sizes of proteins made in vitro from RNA isolated from infected cells and the sizes of proteins being synthesized in infected cells. Furthermore, all of the detectable ECV structural polypeptides were being made in infected cells between 18 and 21 h p.i., and many polypeptides of the same size were made in vitro from RNA isolated at 21 h p.i. We conclude that AcMNPV mRNAs were efficiently translated in vitro into proteins whose molecular weights are similar to those of authentic viral proteins. Further identification of in vitro-synthesized proteins will be necessary to confirm that they are identical to viral proteins which have similar molecular weights.

We have used in vitro translation of RNA complementary to purified AcMNPV DNA restriction fragments to map the locations of specific viral mRNAs. Cytoplasmic RNA isolated from infected cells at 21 h p.i. was used in these studies because all of the virion structural proteins, polyhedrin, 10K, and at least 10 other virus-specific proteins were being synthesized at this time. One of the more striking features about the organization of AcMNPV genes was that many of the genes for the major structural and nonstructural proteins were located in regions that together represent nearly the entire genome (Fig. 6 and Table 1). The locations of AcMNPV genes for two major virion polypeptides, 41K and 64K, as well as those for other virion polypeptides do not appear to be clustered. This suggests that many AcMNPV late genes may be under the control of different promoters. These results are consistent with the observation that in vitro-radiolabeled poly(A)$^+$ RNA isolated at 21 h p.i. hybridized to all AcMNPV *Bam*HI and *Eco*RI DNA restriction fragments (16). In addition, Vlak et al. (16) also observed that sequences homologous to *Eco*RI-P are more abundant than most other sequences present in poly(A)$^+$ RNA isolated at 21 h p.i. In the present report we showed that AcMNPV mRNA specific for this region of the genome was translated into a 10K polypeptide. Therefore, the high level of translation of the 10K polypeptide at 21 h p.i. can likely be correlated with a high steady-state level of the mRNA for 10K.

In a previous report we concluded that the polyhedrin gene was located on fragment *Eco*RI-I (16). We could not exclude the possibility that leader sequences could be responsible for the hybridization of polyhedrin mRNA to *Eco*RI-I. However, none of the other fragments used in this study, which represent the entire AcMNPV genome, selected for polyhedrin mRNA. Within fragment *Eco*RI-I, only mRNA selected from *Hin*dIII-V, but not from adjacent fragments *Hin*dIII-F and -T, could be translated into polyhedrin. Fragment *Hin*dIII-V is about 930 base pairs long, and the mRNA, as detected by northern blotting experiments, has a length of 1,100 to 1,200 bases (Smith et al., in preparation). Since the mRNA for polyhedrin is polyadenylated (16) the actual coding sequences can be estimated to be about 1,000 bases. Therefore, it is likely that *Hin*dIII-V to a large extent contains the coding sequence for polyhedrin. We have additional evidence that the actual gene extends into the adjacent *Hin*dIII-F, but that there are too few complementary sequences to form a stable DNA/RNA hybrid in the hybridization reaction (Smith et al., in preparation). The specific selection of polyhedrin mRNA only from *Hin*dIII-V also demonstrated that there

was one polyhedrin gene copy on *Eco*RI-I and most likely on AcMNPV DNA as a whole.

We do not know whether RNA splicing of non-coding sequences, e.g., 5' leader sequences, was responsible for the hybridization selection of RNAs, which would lead to a misinterpretation as to the location of the coding sequences for particular mRNAs. However, most of the mRNAs mapped in this report were selected for by DNA fragments from only one region of the genome. Two exceptions were mRNAs for 24K and 38K polypeptides, each of which was mapped to two different regions. Although this could be a result of RNA splicing of noncontiguous sequences, it is just as likely that there were AcMNPV genes specifying for two different 24K and two different 38K polypeptides.

Early AcMNPV mRNA isolated at 4 h p.i. was translated in vitro into at least six polypeptides. ICSPs of the same size were detected in cells pulse-labeled 3 to 6 h p.i. In several previous studies it was concluded that AcMNPV proteins induced early in infected cells are also being synthesized at least as late as 21 h p.i. (1, 3, 8, 17). We can speculate that either early mRNAs are being transcribed and translated continuously, or the mRNAs which were made before DNA replication are stable, or both. In any event, many of the mRNAs specific for early proteins are most likely present at 21 h p.i. Those mRNAs, which were present late and have been mapped in the present report, may be the same as those transcribed early. These mRNAs map within two regions of the AcMNPV genome: mRNAs for 25K and 39K polypeptides map between 15 and 22 map units, and the mRNAs for 29K, 32K, and 47K polypeptides map between 45 and 60 map units. Several other important conclusions can be made regarding early transcription: (i) based on a comparison of molecular weights only, none of the early polypeptides become major components of the virus; (ii) the 39K polypeptide was the major early polypeptide, was made at relatively high levels throughout most of the infection cycle, and most likely plays an important regulatory role; (iii) there were no detectable host proteins whose syntheses were increased as a result of viral infection.

In most instances in which quantitative or qualitative changes occur in protein synthesis in eucaryotic cells, there is a corresponding shift in the steady-state levels of specific mRNAs in the cytoplasm. This is also generally true in virus-infected animal cells (for review, see Darnell [2]). In the present report we have demonstrated that changes that occur in protein synthesis in AcMNPV-infected cells early and late in the infection cycle were concomitant with the presence of specific mRNAs, as assayed by in vitro translation of hybridization-selected viral mRNAs. We have also mapped the approximate location of AcMNPV DNA sequences for specific mRNAs present late in infected cells. These results help to define those regions of the genome which will be of interest for more detailed studies in the future.

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