Sequence, Transcriptional Mapping, and Overexpression of p47, a Baculovirus Gene Regulating Late Gene Expression

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A 2.8-kb region of the *Autographa californica* nuclear polyhedrosis virus genome was sequenced and found to contain an open reading frame (p47) which was capable of rescuing a previously characterized temperature-sensitive mutant, *ts317* (S. Partington, H. Yu, A. Lu, and E. B. Carstens, *Virology* 157:91–102, 1990). Transcriptional mapping demonstrated that an early 4.2-kb RNA encoded the p47 open reading frame and probably overlapped the 39K delayed-early gene. The p47 open reading frame was cloned behind the polyhedrin promoter in a baculovirus transfer plasmid, which was then used to prepare a recombinant baculovirus overexpressing the p47 polypeptide. The overexpressed polypeptide was used to prepare p47-specific monoclonal antibodies. These antibodies detected a polypeptide of 47 kDa in *A. californica* nuclear polyhedrosis virus-infected cells, demonstrating that p47 is expressed as an authentic viral product. The p47 gene product was localized to the nucleus of infected cells, supporting the hypothesis that it is involved in regulating viral transcription at late times postinfection.

The *Autographa californica* nuclear polyhedrosis virus (AcMNPV) is the type species of the family *Baculoviridae*, a group of enveloped double-stranded DNA viruses whose replication is restricted to invertebrates. AcMNPV serves as the model baculovirus and, as such, is the best-characterized member of this family.

The regulation of baculovirus gene expression has attracted attention recently because these viruses may prove very useful as biological pest control agents (26). Our research into the replication cycle of baculovirus is directed toward identifying genes which could be genetically manipulated to augment virulence and alter host specificity. This work will complement other research directed toward engineering baculoviruses as effective pest controls agents by introducing into the viral genome genes expressing insecticidal activity (20). Attention has also focused on baculoviruses because they are an efficient eukaryotic expression system (12). For this work, it is important to understand the mechanisms of transcriptional and translational control in insect cells.

We have used a genetic approach to identify and characterize viral genes involved in regulating the virus replication cycle. Several groups have reported the isolation of conditionally lethal temperature-sensitive (*ts*) mutants (2, 6, 17, 22), but the mutated gene has been identified in only one of these, AcMNPV *ts8*. This defect has been mapped to an early gene called p143, which is essential for DNA replication and has homology to helicases (8, 18). We have recently described the isolation and preliminary characterization of two other AcMNPV *ts* mutants, *ts317* and *ts538* (22). These two mutants appear to have defects in genes which are expressed late after infection and are critical for the shift to very late gene expression. Cells infected with either of these mutants shut off viral protein synthesis within 24 h after infection at the nonpermissive temperature. Interestingly, although the phenotypes of these two mutants are very

similar, results of complementation assays indicated that they map to two different genetic loci (22). Since the mutants are also defective in transcribing late viral genes at the nonpermissive temperature, we believe that identifying the defective genes in these mutants will lead to a better understanding of the viral regulatory processes.

In this paper, we describe the physical mapping and nucleotide sequence analysis of the mutation site in *ts317*. Using this information, we have discovered a new baculovirus gene, p47. A 4.2-kb transcript carries the p47 open reading frame (ORF) but also carries other large ORFs including the previously described delayed-early 39K gene (9). Primer extension analysis was used to map the 5' end of the 4.2-kb RNA and identify the promoter region. The p47 ORF was cloned into the pJVETLZ transfer plasmid (25) behind the polyhedrin gene promoter to generate recombinant baculoviruses which overexpressed the p47 polypeptide. P47-specific monoclonal antibodies were generated and used to demonstrate that p47 is an authentic viral gene product. The p47 protein was found in the nucleus of infected cells, suggesting that p47 could be functioning there as a gene regulator.

MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* cells (Sf cells) and AcMNPV HR3 were propagated as previously described (19). The temperature-sensitive mutant of AcMNPV, *ts317* (22), was propagated at 25°C.

Marker rescue. The location of the *ts317* lesion was physically mapped by cotransfection into Sf cells at 25°C of 200 ng of purified *ts317* virus DNA and 1 µg of DNA from a series of plasmids containing different wild-type AcMNPV fragments (8). The 50% tissue culture infective dose of the resulting infected-cell supernatants was determined at 25 and 33°C to identify the region of the wild-type genome that rescued the *ts* mutation. Positive rescue was indicated by the

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presence of polyhedron-producing recombinant virus in cells infected at 33°C.

DNA cloning and sequence analysis. Initial marker rescue results indicated that the *ts317* mutation was located within a region shared by the *EcoRI* J and *HindIII* E fragments of AcMNPV (23.7 to 25.3 map units [m.u.]). To localize the mutation to a smaller region, we prepared subclones by digesting the pUC8 clone pAcEcoJ (5) with *HindIII*, religating, and selecting clones with a 2-kb insert, corresponding to the right end of the *EcoRI* J region. This insert was then cut with *HindIII* and *XhoI*, and the resulting 0.5- and 1.5-kb fragments were isolated and subcloned into pUC19 cut with *SalI* and *HindIII* (pAcEcoJ0.5) or *EcoRI* (pAcEcoJ1.5). Further subclones of pAcEcoJ1.5 were generated by digesting the insert with *SalI* and *EcoRI* to yield pAcEcoJ1.1SE or with *HindIII* and *SalI* to yield pAcEcoJ0.5XS. Finally, pAcEcoJ1.1SE was digested with *HincII* and religated to give pAcEcoJ1.1(0.5). The *EcoRI* J fragment was also cloned from *ts317* viral DNA, and similar subclones were generated so that the region carrying the mutation could be sequenced. The nucleotide sequence of the various clones was determined by double-stranded dideoxynucleotide-sequencing methods with a T7 DNA sequencing kit (Pharmacia Canada) as previously described (18). Both strands were completely sequenced, and each nucleotide was determined at least four times. Searches for nucleic acid and amino acid homologies were done by using the data bases available through IntelliGenetics Inc., GenBank Online Service.

RNA purification, Northern (RNA) blotting, and primer extension analysis. Total-cell RNA from uninfected and AcMNPV-infected Sf cells was isolated by the guanidinium isothiocyanate-CsCl method as previously described (19). RNA was denatured by glyoxalation, electrophoresed, transferred to nitrocellulose, and hybridized with either DNA or cRNA probes. DNA probes were prepared by labeling DNA fragments with [α - 32 P]dCTP by using a random primer kit as specified by the manufacturer (Pharmacia Canada). To facilitate the production of strand-specific RNA probes, a 949-bp *EcoRI-SalI* fragment from the *EcoRI* J fragment was cloned into the plasmid vector pGEM-3, resulting in the construct pGEMES0.9. Strand-specific RNA probes were synthesized by using either T7 or Sp6 RNA polymerase in the presence of [α - 32 P]CTP and pGEMES0.9 linearized with *EcoRI* or *HindIII*.

Primer extension analysis was conducted by using a γ - 32 P-end-labeled 15-base oligonucleotide primer (5'-CAGTTTGCACGCTTG-3') complementary to the sequence +43 to +57 relative to the AUG codon of the p47 gene. Annealing and extension reactions were performed as described previously (19) with 110 pmol of primer annealed at 45°C to 25 μ g of total-cell RNA isolated at various times postinfection (p.i.). Extension products were identified after electrophoresis through denaturing 6% polyacrylamide gels and autoradiography.

Construction of recombinant baculovirus overexpressing p47. The 1.5-kb *XhoI-EcoRI* restriction fragment from AcMNPV, corresponding to map units 24.1 to 25.3 and containing the complete ORF of p47 and the amino-terminal region of ORF2, was subcloned into the single *NheI* cloning site of the pJVETLZ transfer vector (25) to produce pJVETLZp47. The presence of the correct insert was confirmed by sequence analysis. The recombinant virus Acp47 was constructed by cotransfecting 2 μ g of pJVETLZp47 and 1 μ g of AcMNPV DNA into Sf cells by the calcium phosphate precipitation method (3). Recombinant viruses were isolated from the transfection supernatant by plaque purifi-

cation in the presence of 100 μ g of Blue-Gal (GIBCO-BRL) per ml. Blue plaques, representing recombinant viruses, were then picked and plaque purified. Pass 3 stock of the recombinant virus was used in subsequent experiments.

Preparation of cell extracts and Western blotting. Monolayers of Sf cells were infected with wild-type virus or Acp47 (multiplicity of infection, 10) and incubated at 28°C. To radioactively label newly synthesized proteins at various times p.i., the infected cells were washed twice with methionine-free medium and radiolabeled for 1 h with 100 μ Ci of [35 S]methionine per ml in methionine-free medium. The labeled cells were then centrifuged, washed in phosphate-buffered saline (PBS), resuspended in electrophoresis sample buffer, and boiled for 5 min. The protein samples were separated by electrophoresis on sodium dodecyl sulfate (SDS)-8% polyacrylamide gels (16). The gels were then either stained with Coomassie blue, dried, and exposed to X-ray film or prepared for Western immunoblotting by being transferred to nitrocellulose membranes as previously described (22). Hybridoma tissue culture supernatant or ascitic fluid containing anti-p47 antibody was used as the primary antibody. Alkaline phosphatase-conjugated anti-mouse immunoglobulin G goat antibody (Sigma) was used as the secondary antibody.

To determine the intracellular localization of certain proteins, we also prepared cytoplasmic and nuclear extracts of infected cells (13) and analyzed them by SDS-polyacrylamide gel electrophoresis.

Partial purification of p47 and production of p47-specific monoclonal antibodies. Sf cells were infected with Acp47 recombinant virus (multiplicity of infection, 10), and at 48 h p.i. the nuclear fraction was isolated as described above. The protein sample was electrophoresed on an SDS-8% polyacrylamide gel, and the region of the gel containing the 47-kDa band was excised, crushed, resuspended in 10 mM NaHCO₃-Na₂CO₃ (pH 9.6) (carbonate buffer), and eluted by passive diffusion. The eluted polypeptides were precipitated in 8 volumes of ethanol, pelleted by centrifugation at 3,000 $\times$ g, resuspended in carbonate buffer, and used for monoclonal antibody production. BALB/c mice were immunized and boosted with 500 μ l of p47 (10 μ g/ml) and Quil A adjuvant (20 μ g/ml). Injections were given intraperitoneally at 1-week intervals. Once maximal titers of p47 antisera were reached, as determined by enzyme-linked immunosorbent assays (ELISA), the spleens were removed and homogenized in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine albumin (DMEM complete medium) by using a Dounce homogenizer. Murine myeloma SP2/0 cells were added to the spleen cells at a ratio of 10:1. The cells were centrifuged at 1,500 $\times$ g for 10 min at 4°C, resuspended in 0.8 ml of 50% polyethylene glycol in DMEM, incubated at room temperature for 5 min, and then slowly diluted with 10 ml of DMEM. The fused cells were incubated at 37°C for 20 min and centrifuged. The cell pellet was resuspended in 50 ml of DMEM complete medium containing 10 mM sodium hypoxanthine and 1.6 mM thymidine, aliquoted into six 24-well plates containing murine BALB/c feeder cells, and incubated for 16 h at 37°C. DMEM containing 10 mM sodium hypoxanthine, 40 mM aminopterin, and 1.6 mM thymidine was then used to select for hybridomas. Hybridomas were screened by testing the cell culture supernatant by ELISA with purified p47 protein as the antigen. Biotinylated goat anti-mouse immunoglobulin G antibody (Amersham), diluted 1:1,000 in PBS containing 0.01% Tween-20 (BDH), was used as the secondary antibody. The colorimetric enzyme, biotinylated streptavidin-conjugated

FIG. 1. Marker rescue and sequencing strategy. The relative positions, sizes, and map locations of various cloned DNA fragments of AcMNPV which were capable of rescuing *ts*317 are shown below the *Eco*RI restriction fragment map of AcMNPV. The smallest fragment rescuing the mutation was an *Hinc*II-*Eco*RI fragment mapping to the right end of the *Eco*RI J fragment (24.9 to 25.3 m.u.). The bottom of the figure shows the location and direction of sequences determined from this region. The *Pst*I site at the left end of the sequenced region corresponds to the right end of the previously sequenced *Pst*I J fragment (9). Abbreviations: P, *Pst*I; H, *Hind*III; X, *Xho*I; S, *Sall*; Hc, *Hinc*II; E, *Eco*RI.

horseradish peroxidase (Amersham), was used at a 1:1,000 dilution. The color substrate (50 mM Na_2PO_4 , 25 mM citric acid, 0.025% H_2O_2 , 9.25 mM *o*-phenylenediamine) was added and allowed to react until the color change reached maximum levels. Hybridoma cells within positive wells were cloned by limiting dilution and rescreened by ELISA and Western blotting.

Murine ascitic fluid containing p47-specific monoclonal antibodies was also produced. BALB/c mice were given an intraperitoneal injection of 0.5 ml of 2,6,10,14-tetramethylpentadecane (Sigma) followed 7 days later by a second intraperitoneal injection of 2×10^5 p47-specific monoclonal antibody-producing hybridoma cells resuspended in 0.5 ml of DMEM complete medium. Once the tumor was visible, the ascitic fluid was harvested, its titer was determined, and it was used in immunodetection assays at 10^{-4} dilutions.

Nucleotide sequence accession number. The nucleotide sequence of the AcMNPV p47 gene has been deposited with GenBank under the accession number L07878.

RESULTS

Gene mapping and sequence analysis. Marker rescue was used to locate the gene responsible for the phenotypic changes seen in *ts*317-infected cells at the nonpermissive temperature. Initial experiments mapped the *ts*317 mutation within a region overlapping the *Eco*RI J and *Hind*III E fragments (23.7 to 25.3 m.u.). By subcloning portions of this region and using the cloned fragments in further marker rescue experiments, we mapped the *ts*317 mutation to a 464-nucleotide (nt) *Hinc*II-*Eco*RI fragment located at the right end of *Eco*RI J (24.9 to 25.3 m.u.) (Fig. 1).

To determine the primary structure of this region and identify the affected gene, a 2768-nt region of AcMNPV, extending from the left end of the *Pst*I F restriction fragment to 357 bp past the right end of the *Eco*RI J fragment (23.4 to 25.7 m.u.) was sequenced (Fig. 1). In addition, the 464-nt *Hinc*II-*Eco*RI fragment that contained the *ts*317 mutation was cloned and sequenced. We also sequenced through the *Pst*I site at 23.4 m.u. Therefore, our sequence adjoins the

AcMNPV *Pst*I K sequence previously published (9). Two large ORFs were found within this region (see Fig. 4), oriented in an anticlockwise direction with respect to the conventional virus map. The p47 ORF contains 1,206 nt (401 amino acids) and has a predicted molecular mass of 47.5 kDa. Located close to the p47 termination codon is a second ORF (ORF2) of 1,092 nt that encodes a predicted protein of 363 amino acids with a molecular mass of 43.5 kDa. These ORFs are separated by 8 bp. Both of the methionine codons at the beginning of the ORFs conform well to Kozak's rules for optimal translation efficiency, having a purine at position -3 (14). A single nucleotide change (T for C) in the p47 ORF was found in the *ts*317 sequence (Fig. 2, nt 2001), resulting in the incorporation of a methionine in the place of a valine. Computer searches revealed no nucleic acid or amino acid sequence with significant similarity to p47 or ORF2 (GenBank release 72, EMBL release 31, SWISS-PROT release 22, and GenPept release 72).

Transcriptional analysis of the 22.3- to 25.3-m.u. region of AcMNPV. We have previously shown that the major transcript expressed by the *Eco*RI J region was an early transcript of about 1.3 kb (7). To determine the transcription patterns from the p47 and ORF2 regions, Northern blots containing total-cell RNA from AcMNPV-infected cells were probed with *Hind*III-R (22.3 to 23.6 m.u.) DNA. Five transcripts of 4.6, 4.2, 3.1, 1.7, and 1.3 kb were detected at various times p.i., although the early 1.3-kb transcript was the most abundant (Fig. 3). This transcript, seen at 3 h p.i. and present maximally at 12 h p.i., probably codes for the 39K delayed-early gene (9). A 4.2-kb transcript was also present at 3 h p.i. but at greatly reduced levels compared with the 1.3-kb transcript. It was also present at 9 and 12 h p.i. but was not detectable at 24 h p.i. A 1.7-kb transcript was first seen at 6 h p.i. and peaked at 9 h p.i. These results suggested that the 1.3- and 4.2-kb transcripts were early transcripts. The early expression of these transcripts was confirmed by detection of homologous transcripts in AcMNPV-infected cells in the presence of cycloheximide and in *ts*8-infected cells at the nonpermissive temperature (Fig. 3).

FIG. 2. Sequence of the right end of the AcMNPV *EcoRI* J fragment. The nucleotide sequence is oriented in a clockwise direction with respect to the standard AcMNPV map. The predicted amino acid sequences of the two largest ORFs, p47 and ORF2, are shown below the DNA sequence. The location of the 4.2-kb RNA transcription starts sites have double underlines, and the specific start nucleotides are indicated (). The nucleotide alteration in *ts317* is shown at position 2001. The location of the primer used in primer extension analysis is indicated by the dotted underlining.

We then investigated the possibility that the 4.2-kb transcript coded for p47 or ORF2 by identifying regions of viral DNA which hybridized with this transcript.

Three DNA fragments were radiolabeled and used to probe Northern blots of RNA isolated at 6, 9, 12, and 24 h p.i. (Fig. 4). An *XhoI*-*SalI* fragment (Fig. 4, probe A) which overlaps ORF2 and p47 hybridized to the 4.2-kb transcript at 6, 9, and 12 h p.i. The 4.2-kb transcript also hybridized with a *SalI*-*EcoRI* fragment (Fig. 4, probe B) contained entirely within p47 but did not hybridize with a region to the right of the p47 ORF (Fig. 4, probe C). These results indicate that the 4.2-kb transcript overlapped p47, ORF2, and 39K. The very low abundance 3.1-kb transcript was detected by the *SalI*-*EcoRI* (probe B) and *EcoRI*-*SalI* (probe C) fragments but was not investigated further.

Single-strand-specific cRNAs were used to probe Northern blots to determine the direction of transcription of the 4.2-kb transcript (Fig. 5). A cRNA probe designed to detect transcripts in the counterclockwise direction on the circular genome hybridized to the 4.2-kb RNA (Fig. 5, probe A) whereas no transcripts were detected with a cRNA probe

designed to detect RNA transcribed in the opposite direction (Fig. 5, probe B). These results indicate that the 4.2-kb RNA is transcribed in a direction consistent with the orientation of the p47, ORF2, and 39K genes.

Mapping of the 5' end of the 4.2-kb transcript. The transcriptional start site for the 4.2-kb transcript was mapped by primer extension analysis. A 15-nt primer complementary to +43 to +57 relative to the translational start codon of p47 was annealed to total-cell RNA isolated at various times p.i. (Fig. 6). Extension products of 108, 143, and 144 nt were observed. The 108-nt product was seen at 3 (after long exposure), 6, and 9 h p.i. and mapped to the first G within a 5'-CGTGC-3' motif. The 143- and 144-nt products were also present at these times but continued to be produced at 12 and 24 h p.i., although maximal expression occurred between 9 and 12 h p.i. The 143- and 144-nt products correspond to the T and A, respectively, of a 5'-GTAAAC-3' motif (Fig. 2). The initiation sites determined for the 4.2-kb RNA suggest that the 3' end of the 4.2-kb RNA would terminate very close to a poly(A) addition signal located at the carboxyl-terminal region of 39K (9).

FIG. 4. Northern blot analysis of AcMNPV transcripts in the region of the delayed-early 39K gene. RNA purified from AcMNPV-infected cells at various times p.i. was separated on denaturing agarose gels, blotted onto nitrocellulose filters, and hybridized with cloned fragments of viral DNA overlapping ORF2, the p47 ORF, and sequences to the right of the p47 ORF. The results suggest that the 4.2 kb that overlaps 39K also overlaps ORF2 (probe A) and the p47 ORF (probe B) but not sequences further to the right of this region (probe C). The location of the 3.1-kb transcript was not determined.

virions, both extracellular and polyhedron derived, were negative, suggesting that p47 was not incorporated into virions in detectable amounts (4). Taken together, these results support our previous hypothesis that p47 is a baculovirus regulatory protein functioning in the nucleus at the level of transcription (22). These results also confirm that p47 is an authentic viral product and that its synthesis in infected cells corresponds well to the expression of the 4.2-kb RNA, which was also maximally expressed by 9 h p.i.

DISCUSSION

Marker rescue of the temperature-sensitive lesion in *ts317* and the subsequent nucleotide sequence analysis of that region of AcMNPV led to the discovery of a baculovirus gene which we have called p47. This gene is encoded by a 4.2-kb transcript which extends in a counterclockwise direction on the standard AcMNPV map and encodes other large ORFs including the previously described delayed-early 39K gene (9). The 4.2-kb transcript initiates to the right of the p47 gene and was found to completely overlap the 39K gene. It probably shares coterminal 3' ends with the 39K 1.3-kb transcript. Since the AUG of p47 is the proximal initiation codon to the 5' end of the 4.2-kb transcript, p47 is probably the only gene product produced by this transcript.

Northern blot analysis indicated that the 4.2-kb transcript was maximally expressed between 6 and 12 h p.i., after the onset of DNA replication (23), suggesting that this transcript belongs to the late (gamma) class of baculovirus RNAs. However, this transcript was also expressed earlier, in the presence of cycloheximide, and in *ts8*-infected cells at the nonpermissive temperature (Fig. 3). This suggested that the

FIG. 5. Direction of transcription of the 4.2-kb RNA. Strand-specific probes were prepared by cloning the *SalI-EcoRI* 1.2-kb fragment at the right end of the *EcoRI* J region into pGEM3 as described in Materials and Methods. In vitro-synthesized [32 P] cRNA was used to probe Northern blots of RNA from AcMNPV-infected cells at 6, 9, 12, and 24 h p.i. Only the probe consistent with a leftward transcript (A) hybridized with the 4.2-kb transcript. No transcripts hybridized with the opposite strand probe (B).

4.2-kb transcript was also expressed as an early transcript. Primer extension data supported this hypothesis and also revealed that the 4.2-kb RNA has two transcriptional start sites. A start site mapping within a 5'-CGTGC-3' motif was used only prior to 9 h p.i., whereas a second start site within a 5'-GTAAAC-3' motif produced transcripts which could still be detected at 24 h p.i. The fact that this second start site does not conform well to the baculovirus late consensus transcription initiation sequence may explain the low abundance of this transcript at late times. The 5'-CGTGC-3' motif has been found at the early start sites of the p143 gene (19) and the DNA polymerase gene (24). Transcription of the 4.2-kb RNA initiating from this site is probably responsible for its cycloheximide resistance and its presence in *ts8*-infected cells at 33°C, as has previously been shown for the p143 transcript (19). The 39K transcript also possesses dual RNA start sites, which allow it to be expressed prior to DNA replication and at late times p.i. (9). However, the 39K early start site initiates within a 5'-CAGT-3' motif whereas the late start site initiates within a 5'-ATAAG-3' sequence.

By overexpressing the p47 ORF in a recombinant baculovirus, we demonstrated that the sequence analysis was correct in predicting a polypeptide of 47 kDa being encoded by this region of the AcMNPV genome. In addition, since

FIG. 6. Primer extension analysis of the 4.2-kb transcript. RNA harvested from AcMNPV-infected cells at various times p.i. was analyzed by primer extension reactions. Primer extension products were separated on denaturing sequencing gels. Three major extension products were seen, one at 3 to 9 h p.i. (108 nt) and two which were present between 3 and 24 h p.i. (143 and 144 nt). The bands at 3 and 24 h p.i. were seen after longer exposures. The locations of these transcript start sites are indicated in Fig. 2.

the recombinant virus carried an additional 300 nt of DNA corresponding to about 100 amino acids from ORF2, the fact that the expressed product was of the predicted size confirmed the location of the stop codon predicted for p47. The overexpressed p47 also allowed us to prepare monoclonal antibodies directed against this polypeptide. With these

FIG. 7. Time course of protein synthesis following infection with a recombinant baculovirus overexpressing p47. Sf cells were labeled with [35 S]methionine at various times p.i. and analyzed by SDS-polyacrylamide gel electrophoresis. This autoradiogram shows the hyperexpression of a 47-kDa polypeptide in recombinant virus-infected cells (lanes Acp47) at approximately the same time and to the same level as polyhedrin in AcMNPV-infected cells (lanes wt).

FIG. 8. Localization of p47 in infected Sf cells. Immunoblots were prepared from extracts of 2×10^5 mock-infected (lanes M) or AcMNPV-infected (lanes Wt) cells and 1×10^5 Acp47-infected cells (lanes p47) harvested at 24 h postinfection. Whole-cell (lanes W), nuclear (lanes N), and cytoplasmic (lanes C) fractions were prepared to identify the cellular compartment where the p47 gene product accumulated. The blots, incubated with monoclonal antiserum MAb5.9, reveal the accumulation of the p47 polypeptide in the nucleus of both AcMNPV-infected and Acp47 recombinant virus-infected cells.

antibodies, we have conclusively shown that p47 is an authentic viral protein which is synthesized in infected cells (Fig. 8). The time course of expression of the 47-kDa protein in infected cells also correlates well with the temporal expression of the 4.2-kb mRNA, being expressed at very low levels early and being most abundant at 9 to 12 h p.i. However, whereas the 4.2-kb RNA was barely detectable at 24 h p.i., the 47-kDa protein could still be detected in infected cells until at least 72 h p.i. This suggests that the p47 gene product is stable during the very late stages of virus infection.

Our previous analysis of the phenotype of *ts317* suggested that p47 may be involved in regulating the expression of late and very late genes in AcMNPV-infected cells. At the nonpermissive temperature, *ts317*-infected cells produced almost normal levels of viral structural proteins, but the synthesis of these proteins apparently was restricted to the period prior to about 20 h p.i. (23). In addition, small amounts of the very late proteins such as polyhedrin or p10 were detected in *ts317*-infected cells at the nonpermissive temperature. The absence of the very late gene products was probably due to the absence of the transcripts for these genes, suggesting that p47 was required for transcription of very late gene promoters. p47 may be an accessory protein which modifies the activity of a RNA polymerase responsi-

FIG. 9. Temporal expression of the p47 gene product in infected cells. An immunoblot of 2×10^5 AcMNPV-infected cell nuclei (lanes AcMNPV) prepared at the indicated times p.i. and 5×10^4 recombinant virus-infected cells (lane Acp47) prepared at 24 h p.i. was incubated with monoclonal antibody MAb5.9. The migration location of the p47 gene product is indicated on the right.

ble for transcription initiation at certain late and very late start sites (11, 22). It is interesting that although the sequence (A/G)TAAG has been recognized as a consensus transcription initiation site for late and very late baculovirus genes (1), other factors must be involved in differentiating these promoters to allow for the hyperexpression of some genes such as polyhedrin and p10. To date, only the consensus sequence 5'-AATAAGTATTTT-3' at the transcription initiation site has been correlated with hyperexpressed late genes (1, 10, 15). However, it is possible that there are other families of late genes which are differentially regulated, some of which may code for structural proteins while others may code for regulatory proteins. Further work is necessary to determine the exact role of p47 in regulating late-gene expression. We are currently investigating the ability of p47 to form a protein complex which could bind DNA, specifically to late and very late viral promoter sequences.

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