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Identification of the Very Early Transcribed Baculovirus Gene PE-38

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We have started to identify early viral RNAs that are transcribed at 1 h after inoculation to investigate the mechanism involved in the regulation of early gene expression of *Autographa californica* nuclear polyhedrosis virus (AcNPV). Cloned viral DNA fragments were hybridized to Northern (RNA) blots of polyadenylated RNA isolated from *Spodoptera frugiperda* cells at 1, 2, and 6 h postinfection to localize very early transcripts. Subsequently we prepared a cDNA library of polyadenylated RNA transcribed at 1 h after inoculation to analyze the cDNA clones corresponding to the major early RNAs. We identified a gene located upstream of the immediate-early gene IE-N extending in the opposite direction. Because of the very early expression during AcNPV infection and the transient expression in uninfected cells, we conclude that we found an immediate-early gene, designated PE-38. The determination of the nucleotide sequence of PE-38 revealed one open reading frame potentially encoding a gene product of 38 kDa. Results of in vitro translation experiments suggest that a PE-38-specific polypeptide of approximately 38 kDa can be expressed. We have evidence from computer analyses that the predicted amino acid sequence includes two putative DNA-binding motifs, a zinc finger, and a leucine zipper.

The baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) is a large (128-kb) DNA virus whose transcriptional pattern is characterized by the generation of multiple overlapping RNAs that may be of different temporal classes (16, 27, 29, 34, 38). RNA splicing has so far been described only for the immediate-early gene IE-1 (8). The regulation of gene expression occurs in a sequential fashion and can be temporally subdivided into immediate-early, early, late, and very late phases (24; for reviews, see references 11, 15, and 17). Transcripts of each temporal phase have been mapped throughout the genome, indicating the absence of clustered early or late genes.

At present, the molecular mechanism by which temporal regulation of gene expression occurs is poorly understood. RNA polymerases are thought to be involved in transcriptional switching because early transcription is mediated by a host RNA polymerase II, but an α-amanitin-resistant RNA polymerase is responsible for the majority of late viral transcription (19).

As shown for other large DNA viruses, transcriptional regulation depends on the interaction of *trans*-acting viral factors with *cis*-acting viral DNA elements. Previous studies have demonstrated that early gene products of AcNPV are involved in the transcriptional regulation of later classes of viral genes, but the characterization of these genes is still at an early stage (12, 31, 39). Recently, the ETL gene product has been implicated with the timely expression of other viral genes, though the regulatory delay may be due to a delay in DNA replication (10, 36). Transient expression assays have identified two immediate-early genes, IE-1 and IE-N, stimulating expression of the delayed-early gene 39K by *trans* activation (7, 22). The IE-N gene product has been proposed to participate in the IE-1-mediated activation of the 39K gene when IE-1 is present in limiting amounts (7). In addition, the homologous regions of the AcNPV genome (9) are shown to function as enhancers in the presence of IE-1 (23).

We have started to investigate the sequential and temporally regulated gene expression of AcNPV by analyzing the first genes to be transcribed in order to identify regulatory proteins required for the activation of early and/or late gene transcription.

This report describes the identification of one of the major early transcripts of about 1,300 nucleotides (nt) in length present as early as 1 h after inoculation. We prepared a cDNA library from polyadenylated RNA transcribed at 1 h postinfection (p.i.) to analyze a cDNA clone of 1,075 nt corresponding to the early 1,300-nt transcript. The expression of immediate-early genes does not require previous viral protein synthesis and is thought to be controlled by host factors. Because of the very early expression during AcNPV infection and the transient expression in uninfected *Spodoptera frugiperda* cells, we conclude that we identified an immediate-early gene. This gene was designated PE-38. The site of transcriptional initiation of PE-38 is located about 340 nt upstream of the start site of IE-N, extending in opposite directions.

MATERIALS AND METHODS

Cells and virus. *S. frugiperda* IPLB21 (SF21) cells (50) were grown as monolayer cultures in TC100 medium (20) supplemented with 10% fetal bovine serum. The isolation of AcNPV plaque isolate E, the propagation of the isolate, and the isolation of its DNA were described earlier (49). For studies on the time course of infection, monolayers of cells were inoculated with AcNPV at a multiplicity of 10 to 20 PFU per cell. The AcNPV inoculum was allowed to adsorb for 1 h at 27°C and was then removed and replaced by medium. Time zero was defined as the time when the inoculum was added to the cells.

Plasmid constructions. The *Hin*dIII F fragment, 96.5 to 3.2 map units (m.u.), of AcNPV isolate E was cloned into pBR322. Several subclones of *Hin*dIII-F were generated.

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The BglII K fragment was inserted into the compatible *Bam*HI site of pBluescriptKS(+), thereby destroying both the *Bam*HI and *Bgl*II sites (pAcBgl-K). Similarly, the *Bgl*II-*Eco*RI fragment was cloned into pBluescriptKS(+) digested with *Bam*HI and *Eco*RI (pAcBE). The subclone pAcPE was constructed by ligation of the *Pst*I-*Eco*RI fragment and pBluescriptKS(+) digested with *Pst*I and *Eco*RI.

Transcriptional mapping. At various times after inoculation, infected cells were harvested, and cytoplasmic RNA was isolated by the hot phenol method (42). Polyadenylated [poly(A)$^+$] RNA was selected on oligo(dT)-Sepharose columns (1) and analyzed by electrophoresis on 1.2% agarose gels containing 2.2 M formaldehyde. RNA was heat denatured for 10 min at 65°C before electrophoresis. Northern (RNA) blot analyses were performed with 5 to 10 μg of poly(A)$^+$ RNA per lane. AcNPV-specific RNAs were visualized by hybridization to AcNPV DNA, cloned viral DNA fragments, or strand-specific RNA probes. The transcripts used as RNA probes were synthesized in vitro from the *Bgl*II K fragment cloned into pBluescriptKS(+) by using T3 or T7 RNA polymerase and radioactive UTP (32, 33, 47). When the T3 RNA polymerase was used, the *Bgl*II-K-containing plasmid (pAcBgl-K) was linearized with *Sca*I (see Fig. 3). Prior to in vitro transcription by T7 RNA polymerase, pAcBgl-K was cleaved with *Eco*RI. After removal of the DNA template, the RNAs were used as probes under hybridization conditions described elsewhere (52). The washing temperature of the filters was raised to 75°C to reduce background and to increase stringency.

The 5′ end of the PE-38 transcript was mapped by S1 nuclease protection analysis (3). Plasmid pAcPE was linearized with *Bgl*II, terminally labeled by [γ-^{32}P]ATP and polynucleotide kinase, and finally digested with *Pst*I.

The 179-nt *Bgl*II-*Pst*I fragment was annealed at 45°C to 5 μg of poly(A)$^+$ RNA isolated 1 h p.i. The hybrids were treated with 200 U of S1 nuclease for 30 min at 30°C and analyzed by electrophoresis on a 6% polyacrylamide sequencing gel containing 7 M urea.

Sequencing and computer analyses. The nucleotide sequences of the cDNA clone PE-38 and of the genomic fragments *Bgl*II-K, *Pst*I-*Eco*RI, and *Bgl*II-*Eco*RI were determined by the chain termination method (41), using the T7 sequencing kit (Pharmacia). For the sequencing of either strand of the double-stranded template, 12 synthetic oligodeoxyribonucleotides of 17 nt in length, T7 17-mer, and T3 17-mer (Stratagene) were used as primers.

The DNA sequence and the deduced amino acid sequence of the open reading frame (ORF) were compared with sequences in the updated GenBank/EMBL and SWISS PROT data bases, using the FASTA program (37). The analyses were performed by EMBL (Heidelberg, Federal Republic of Germany).

cDNA synthesis and cloning. cDNAs were synthesized from poly(A)$^+$ RNA isolated 1 h p.i. by using the ZAP-cDNA synthesis system (Stratagene). The primer used for the synthesis of the first strand contains a poly(dT) region and a *Xho*I restriction site [5′-(GAGA)$_5$ACTAGTCTCG AG(T)$_{17}$-3′]. After ligation of *Eco*RI adaptors to the blunt ends of the cDNA and after *Xho*I digestion, the cDNA was size fractionated. cDNA fragments larger than 900 bp were ligated to the Uni-ZAP XR vector arms (Stratagene) and packaged with the Gigapack Gold packaging extracts (Stratagene). Recombinant phages were screened by plaque hybridization with total AcNPV DNA, the *Hin*dIII F fragment, and the *Pst*I-*Eco*RI subfragment. The inserted cDNA fragments were subcloned by in vivo excision of pBluescriptSK(−) from the lambda vector Uni-ZAP XR (43). Analyses of the insert DNA included standard techniques such as Southern blotting (45), nick translation (40), and DNA-DNA hybridization experiments (52).

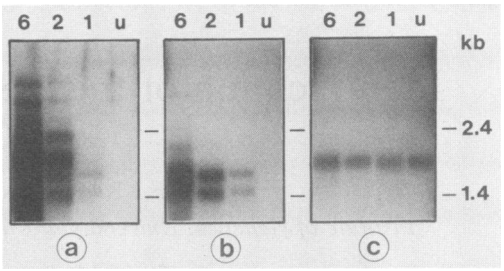

FIG. 1. Northern blot analysis of AcNPV-specific RNA at early times postinfection. Polyadenylated RNA was isolated from uninfected (lane u) and from AcNPV-infected cells at 1 h (lane 1), 2 h (lane 2), and 6 h (lane 6) p.i., electrophoresed on an 1.2% agarose gel, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled AcNPV DNA (a). The filter was reprobed successively with the *Drosophila* actin probe pAct-5c (c) and the cloned *Hin*dIII F fragment (b). Identical samples (5 μg per lane) of polyadenylated RNA were loaded. Positions of the coelectrophoresed RNA ladder (Bethesda Research Laboratories) are indicated on the right. The time of exposure for the autoradiograms was 6 days (a) or 5 h (b and c).

In vitro translation in a reticulocyte lysate system. The PE-38-specific RNA, synthesized and capped in vitro (33), was in vitro translated by standard methods (13). The rabbit reticulocyte lysate system was purchased from Stratagene or Bethesda Research Laboratories. ^{35}S-labeled polypeptides were resolved on 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gels as described previously (25).

Transfection of *S. frugiperda* cells. *S. frugiperda* cells were transfected with the cloned DNA fragments *Hin*dIII-F and *Pst*I-*Eco*RI according to published procedures (21).

RESULTS

Transcriptional analysis of early viral RNAs. We isolated RNA from uninfected and infected *S. frugiperda* cells 1, 2, and 6 h p.i. in order to determine the number and size classes of AcNPV-specific transcripts produced very early after inoculation. The poly(A)$^+$-selected RNA was analyzed on Northern blots by using nick-translated AcNPV DNA (Fig. 1a). As early as 1 h p.i., two RNA size classes of approximately 1.3 and 1.5 kb were observed which became more abundant at 2 and 6 h p.i. In the course of infection (1, 2, and 6 h p.i.), larger transcripts appeared sequentially, ranging from approximately 1.3 to 4 kb at 2 h p.i. and extending at 6 h p.i.

The two major early RNA size classes of 1.3 and 1.5 kb were identified by hybridization of RNA on Northern blots to cloned viral DNA fragments. The *Hin*dIII F fragment (96.5 to 3.2 m.u.) hybridized to both RNA size classes (Fig. 1b). Subclones of the *Hin*dIII F fragment (see Fig. 3b) were used in further hybridization experiments. Both the 1.3- and 1.5-kb RNAs were detected by the *Bgl*II K fragment, but only the smaller transcript hybridized to the *Pst*I-*Eco*RI fragment (data not shown). These results suggest that at least one of the transcripts corresponds to the region of the IE-N gene, which has previously been characterized by Carson et al. (7), and that a further transcript is initiated upstream of the IE-N gene. As a control, the RNA (Fig. 1a and b) was rehybridized to plasmid pAct-5c, containing the *Drosophila*

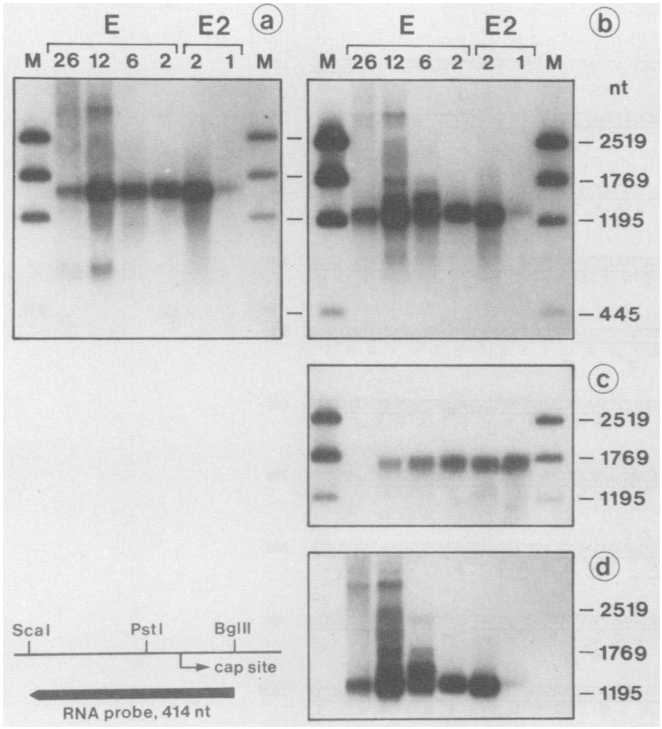

FIG. 2. Northern blot analysis of PE-38- and IE-N-specific transcripts. Polyadenylated RNA was isolated from *S. frugiperda* cells infected with AcNPV plaque isolates E and E2 at 1 h (lane 1), 2 h (lane 2), 6 h (lane 6), 12 h (lane 12), and 26 h (lane 26) p.i. The RNA was electrophoresed on an 1.2% agarose gel, transferred to a nitrocellulose filter, hybridized to the cDNA clone IE-N (a), and rehybridized to the cDNA clone PE-38 (b), to the *Drosophila* actin probe pAct-5c (c), and to an RNA probe of 414 nt complementary to the 5′ part of the PE-38 gene (d). Positions of the DNA size markers (lane M) are indicated on the right.

melanogaster actin 5c gene (5). An actin-specific transcript of about 1.7 kb was present in both uninfected and infected cells (Fig. 1c). The level of transcription seemed to be unaffected by AcNPV infection until 6 to 12 h p.i. (Fig. 1c and 2c). Transcription of the actin gene was, however, switched off late after infection (Fig. 2c). These findings confirm the data published by Ooi and Miller (35).

Identification and analysis of cDNA clones corresponding to the early 1.5- and 1.3-kb RNAs. To identify the two *Bgl*II-K-specific early transcripts, we constructed a cDNA library from poly(A)$^+$ RNA isolated 1 h p.i. The cDNA library was screened with total AcNPV DNA, the purified *Hin*dIII F fragment, and the *Pst*I-*Eco*RI subfragment. A screen of about 2.4×10^4 phage plaques yielded 241 AcNPV-positive clones, as revealed by colony hybridization. The two recombinant phages 26 and 125, hybridizing to *Hin*dIII-F and *Bgl*II-K, were selected for further investigations.

The 5′ end of the 1.3-kb insert of the cDNA clone 125 was sequenced and compared with the nucleotide sequence of the IE-N gene, indicating that cDNA clone 125 represented the polyadenylated 1.5-kb IE-N transcript. In the text that follows, cDNA clone 125 is therefore designated cDNA clone IE-N.

The 1.1-kb insert of cDNA clone 26 was mapped to the *Pst*I-*Eco*RI region and corresponded to the early 1.3-kb transcript, as shown by hybridization experiments (Fig. 2b).

TABLE 1. Summary of the appearance of PE-38 and IE-N transcripts in the course of infection

Time (h) p.i.	Approx size (kb) of polyadenylated RNAs[a]	
	PE-38 transcripts	IE-N transcripts
1	1.3	1.5
2	**1.3**	**1.5**
6	**1.3 1.45** 1.75	**1.5**
12	**1.3 1.45** 1.75 2.0 2.5 3.0 3.3	**1.5** 1.75 2.0 2.5 3.0 3.3
26	1.3 3.3	1.5 3.3

[a] Results of three independent hybridization experiments. Values for the abundant RNAs are in boldface; values for the more weakly expressed RNAs are in lightface.

This major early transcript had not been described previously. As demonstrated in transient expression assays (see below), we identified an immediate-early RNA, whose corresponding cDNA clone 26 is designated cDNA clone PE-38.

Time course of the appearance of PE-38 and IE-N transcripts. We next investigated the transcriptional pattern of the PE-38 and the previously identified IE-N genes in the course of infection. Poly(A)$^+$ RNA from AcNPV-infected cells was prepared at different times postinfection and analyzed on Northern blots. *S. frugiperda* cells were infected with the two AcNPV plaque isolates E (49) and E2 (44), which differ slightly in their restriction patterns, to confirm very early transcription of PE-38 and IE-N in various AcNPV isolates.

The cDNA clone IE-N hybridized to a poly(A)$^+$ RNA of approximately 1,500 nt which was present as early as 1 h p.i., becoming more abundant at 2 to 12 h p.i., and decreasing at 26 h p.i. Late after infection (12 h p.i.), several transcripts were overlapping the IE-N gene. The bulk of these transcripts disappeared at 26 h p.i. (Fig. 2a; details are shown in Table 1).

The data presented in Fig. 2b demonstrate the temporal expression pattern of the PE-38 transcript, which is similar in appearance to the IE-N-specific transcriptional pattern. In addition to the major early 1,300-nt PE-38 RNA, a prominent band of about 1,450 nt was detectable at 6 and 12 h p.i. More weakly expressed RNAs overlapping the PE-38 gene were present late after infection. Table 1 shows a summary of three hybridization studies.

We prepared strand-specific RNA probes to determine the orientation of the PE-38 transcript in comparison with the IE-N transcript. The major early PE-38 RNA and the majority of the overlapping RNAs were transcribed from left to right in opposite directions to the IE-N RNA (Fig. 2d). By using further RNA probes, we could exclude transcription from the antisense strand of PE-38 early after infection (data not shown).

Nucleotide sequence of the PE-38 gene and mapping of the 5′ terminus of the 1,300-nt PE-38 transcript. A series of synthetic primers was used to sequence both strands of cDNA clone PE-38. In addition, the 3′ part of the *Bgl*II K fragment including the promoter of the PE-38 gene was sequenced. The genomic sequence of the PE-38 gene was determined from the *Pst*I-*Eco*RI fragment. The sequence of 1,049 nt and the flanking genomic 5′ and 3′ regions starting with the *Pst*I site are shown in Fig. 3a. Computer analyses revealed one major open reading frame (ORF) of 321 amino acids on the rightward-transcribed strand according to the conventional orientation of the AcNPV genome (51). No

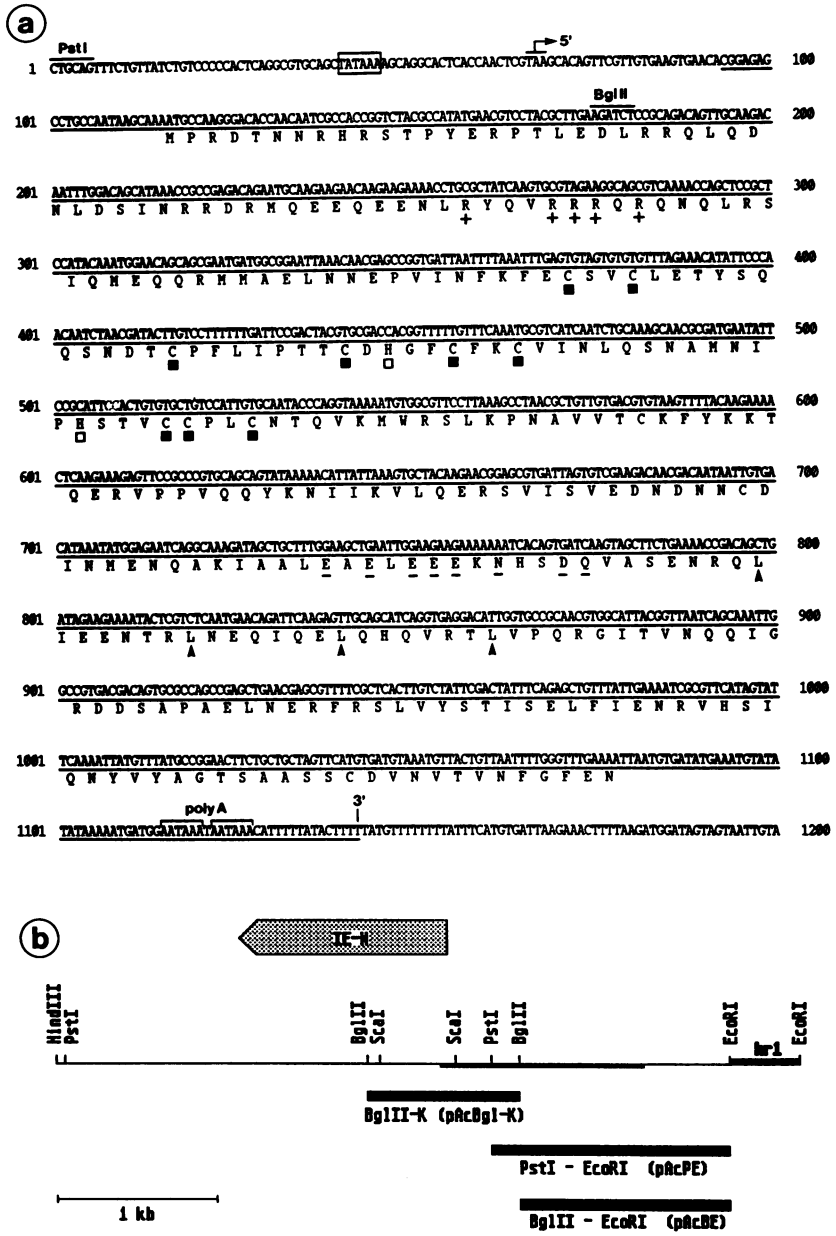

FIG. 3. Nucleotide sequence of PE-38 and the flanking regions. (a) DNA sequence of 1,200 nt that includes the PE-38 gene and part of the flanking sequences. The nucleotides underlined correspond to the cDNA clone PE-38. The predicted amino acid sequence is shown underneath. The 5′ end determined by S1 analysis is indicated by an arrow, and the vertical line at nt 1142 represents the 3′ end of the cDNA clone PE-38. Potential polyadenylation signals are overlined, and a putative TATA signal is boxed. Evaluation of the predicted amino acid sequence is indicated as follows: acidic region by −, basic region by +, periodic repetition of leucine residues by arrows, and the cluster of cysteine and histidine residues by closed or open boxes. (b) Restriction map of the *Hin*dIII (96.5 m.u.)-hr1 (100 m.u.) fragment. The location of the IE-N gene is indicated by an open arrow, and the sequenced fragment is underlined. The subclones are also indicated.

ORF was found on the opposite strand. The stop codon of the major ORF is followed by two closely spaced polyadenylation signals (Fig. 3a).

The 5′ end of the early PE-38 transcript was determined by S1 analysis (Fig. 4). The *Pst*I-*Eco*RI fragment was 5′ labeled at the *Bgl*II site and hybridized to poly(A)$^+$ RNA isolated at 1 h p.i. After treatment with S1 nuclease, the hybrids were electrophoresed on a sequencing gel to map the precise 5′ transcriptional start site. The size of the protected fragment was 113 or 112 nt (Fig. 4), reflecting an early transcriptional start site 48 or 49 nt upstream of the predicted ATG start codon (Fig. 4).

The nucleotide sequences preceding the 5′ terminus of PE-38 carry a TATA-like signal (TATAAA) located 21 to 28 nt upstream of the cap site (Fig. 3a).

The amino acid sequence of the putative ORF is shown in Fig. 3a. A computer search for amino acid sequence homology of PE-38 with other known proteins showed no strong sequence homology with any other protein in the SWISSPROT data base. There is some similarity to regions

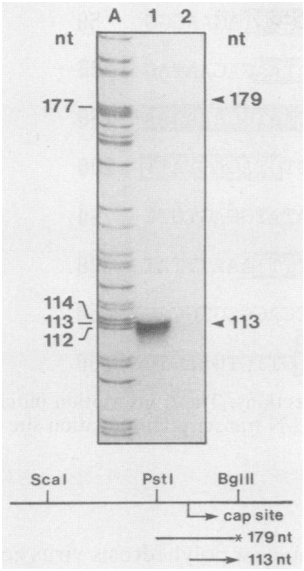

FIG. 4. Mapping of the transcriptional initiation site of the major early PE-38 RNA by S1 analysis. Polyadenylated RNA (5 μg) isolated from AcNPV-infected cells at 1 h p.i. (lane 1) was hybridized to the 5′-labeled *Bgl*II-*Pst*I fragment of 179 nt. The S1 nuclease-resistant hybrids were analyzed on a 6% polyacrylamide sequencing gel. The protected fragment of 113 nt and the localization of the probe are indicated by arrows. As a negative control, 30 μg of tRNA was used in the annealing experiment (lane 2). Sizes of a sequencing ladder (lane A) are indicated on the left.

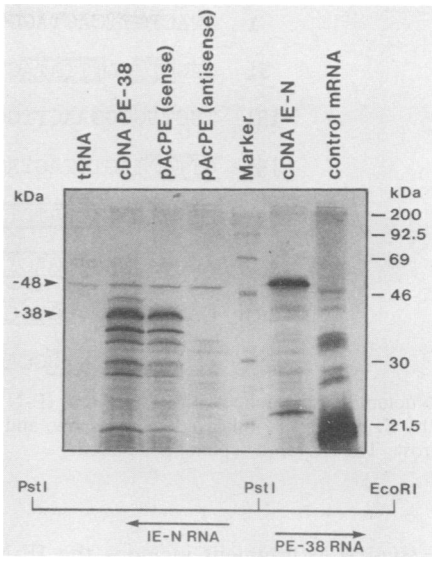

FIG. 5. In vitro translation of the PE-38- and IE-N-specific RNAs. RNA was synthesized in vitro from the cloned *Pst*I-*Eco*RI fragment (pAcPE) in both directions (sense and antisense) and from the cDNA clones PE-38 and IE-N. In vitro translation was performed with approximately 0.5 μg of RNA. As molecular weight markers, a mixture of ^{14}C-labeled proteins (Amersham) was coelectrophoresed. As a control, tRNA and a control mRNA (15 kDa; Stratagene) were translated in vitro. Reaction products were separated on a 12.5% SDS-polyacrylamide gel. The molecular masses of the IE-N- and PE-38-specific polypeptides are indicated on the left, and the sizes of the marker proteins are indicated on the right.

with coiled coil structure in fibrous proteins such as keratin, myosin, and lamin. The corresponding sequence of PE-38 revealed four leucine residues repeating every seventh amino acid (Fig. 3a). The motif of the periodic repetition of leucine residues is referred to as the leucine zipper (26). Further evaluation of the amino acid sequence data revealed a cluster of cysteine and histidine residues at the amino terminus of PE-38 (Fig. 3a) which could form a zinc finger motif (30).

In vitro translation of RNA corresponding to the PE-38 ORF. The PE-38 ORF has the capacity to encode a polypeptide of 321 amino acid residues with a predicted molecular mass of 38 kDa. RNAs synthesized in vitro were translated in a rabbit reticulocyte cell-free translation system to demonstrate the occurrence of a PE-38-specific polypeptide. RNAs were generated in message sense or antisense to the PE-38 gene by using the T3 or T7 promoter of plasmid pAcPE containing the *Pst*I-*Eco*RI fragment. In addition, PE-38 and IE-N sense RNAs were generated by T3 RNA polymerase from the corresponding cDNA clones. A major in vitro translation product of about 38 kDa was observed (Fig. 5) when the two different sense RNAs of PE-38 were used. This value is in good agreement with the predicted molecular mass (38 kDa) of the observed ORF. Additional polypeptide bands of less than 38 kDa were also visible which could represent degradation products. No 38-kDa polypeptide band was generated from the antisense RNA, indicating the specificity of the 38-kDa polypeptide obtained with the message-sense RNAs of PE-38.

In comparison, the IE-N sense RNA was in vitro translated, which led to a single polypeptide of approximately 47 kDa (Fig. 5), slightly different from the size of 43 kDa shown by Carson et al. (7).

Transient expression of the PE-38 gene. We performed transient expression assays to determine whether expression of the PE-38 gene is dependent on viral gene products. The plasmids containing the entire *Hin*dIII F fragment or the *Pst*I-*Eco*RI subfragment were transfected into *S. frugiperda*

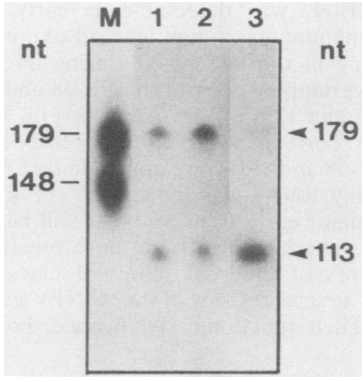

FIG. 6. Initiation of transcription at the early PE-38 cap site in the cloned *Hin*dIII F and *Pst*I-*Eco*RI fragments. Total cytoplasmic RNA was isolated from AcNPV-infected cells at 2 h p.i. (lane 3) and from cells transfected with 10 μg of pAcHind F (lane 1) or pAcPE (lane 2). Approximately 30 μg (lanes 1 and 2) or 20 μg (lane 3) was hybridized to the 5′-labeled *Bgl*II-*Pst*I fragment of 179 nt and analyzed on a 6% polyacrylamide sequencing gel. The 5′-labeled fragments *Bgl*II-*Pst*I (179 nt) and *Acc*I-*Pst*I (148 nt) were coelectrophoresed (lane M). The protected fragment of 113 nt and the 5′-labeled *Bgl*II-*Pst*I fragment of 179 nt are indicated on the right.

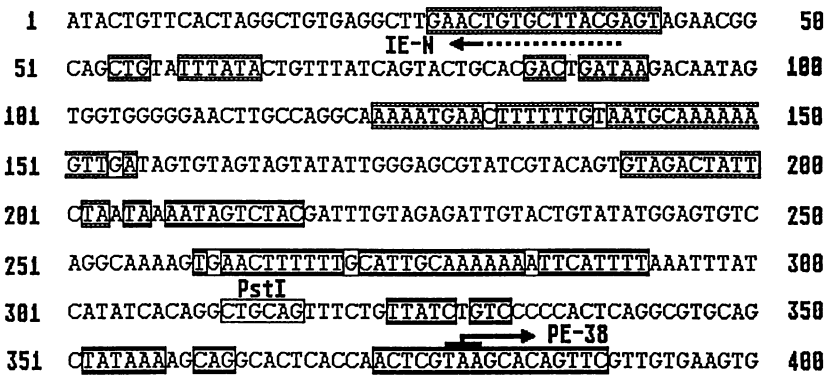

FIG. 7. Nucleotide sequence of the PE-38 and IE-N promoters located in opposite directions. The transcription initiation site of PE-38 determined by S1 analysis is indicated by an arrow, and the approximate location of the IE-N transcription initiation site (7) is indicated by a broken arrow. The inverted repeats are boxed.

cells. The *Hin*dIII F fragment includes the IE-N and the PE-38 genes, whereas the *Pst*I-*Eco*RI subfragment spans only the PE-38 gene. Cytoplasmic RNA was prepared at 48 h after transfection with each of the plasmids, and expression of the PE-38 gene was determined by S1 nuclease analysis. PE-38 was active in *S. frugiperda* cells after transfection of both the total *Hin*dIII F fragment and the *Pst*I-*Eco*RI fragment (Fig. 6). These results suggest that the PE-38 gene was transcribed even in the absence of any other viral protein.

DISCUSSION

We have identified the PE-38 gene corresponding to one of the major early transcripts of AcNPV. This RNA of 1,300 nt in length is located close to the homologous region 1 (9, 23) and is transcribed in a direction opposite that of the previously characterized immediate-early gene IE-N (7).

After cloning very early RNAs from AcNPV-infected *S. frugiperda* cells, we characterized the new PE-38 transcript in comparison with IE-N. The transcription of both RNAs, PE-38 and IE-N, was detectable as early as 1 h after inoculation, maintaining a high level of expression for 12 h and decreasing in the late phase during infection. Larger transcripts overlapping each of the PE-38 and IE-N regions appeared at 6 and 12 h p.i. By using several strand-specific RNA probes corresponding to the sense and antisense strands of PE-38 and IE-N, we conclude that the majority of the overlapping RNAs was located on the same strand as each of the major early transcripts. It will be of interest to localize these overlapping RNAs more precisely. Overlapping transcripts of different temporal classes have been described for several regions of the AcNPV genome (16, 27, 29, 34, 38). Their functional significance, however, is still unclear.

A sequence comparison of the 5′ region of PE-38 with published sequences of early promoters revealed a striking homology to the IE-N promoter (Fig. 7). An inverted repeat of 17 nt includes the cap site of the PE-38 gene and of the IE-N gene as well, separated by 329 nt (Fig. 7). The symmetrical arrangement of inverted repeats within the divergent transcription unit of IE-N and PE-38 is shown in Fig. 7. The significance of these repeats is unclear. The 5′ parts of other early AcNPV genes, such as IE-1, 39K, CG30, ETL, 35K, and 94K (10, 18, 22, 48) as well as of the *Orgyia pseudotsugata* nuclear polyhedrosis virus gene gp64 (4) have no similarity to the inverted repeats of PE-38 and IE-N.

Analysis of the predicted amino acid sequence of the PE-38 ORF revealed two potential DNA-binding motifs. A cluster of cysteine and histidine residues ($CX_2CX_{11}CX_7CXHX_2CX_2CX_2CX_{12}HX_3CCX_2C$) near the amino terminus of the PE-38 gene suggests the possible formation of a zinc finger (30; for reviews, see references 2 and 14). The second putative DNA-binding motif is a leucine zipper (26) located near the carboxy terminus of the PE-38 gene and characterized by the repetition of four leucine residues spaced six amino acids apart. In most cases, basic amino acids are found adjacent to the leucine zipper motif (for a review, see reference 6); PE-38 instead has a basic domain located further away toward the amino terminus. Furthermore, the sequence data indicate a stretch of acidic amino acids close to the leucine repeats (Fig. 3a). At least in yeast and *Escherichia coli* DNA, acidic regions seem to be involved in transcriptional activation (28, 46).

Recently, Thiem and Miller (48) reported that the sequence data of the AcNPV gene CG30 indicated both DNA-binding motifs, the leucine zipper, and the zinc finger. The functional significance of this probably unique feature has yet to be determined.

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