Functional analysis of a 603 nucleotide open reading frame upstream of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus

Katharine L. Gearing and Robert D. Possee*

NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR, U.K.

The sequence of the 2000 nucleotides immediately upstream of the polyhedrin gene of the *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus has been determined. Comparative analysis of the data identified a 603 nucleotide open reading frame (ORF) separated from the polyhedrin gene coding sequences by 156 nucleotides and in the opposite strand of DNA. Northern hybridization analysis of polyadenylated RNA from infected cells highlighted a 3.7 kb species produced maximally at 12 h post-infection, but not in the presence of cycloheximide. Preliminary nuclease S1 analysis of the 5' end of this RNA suggested that it initiated at a position very close to that of the polyhedrin mRNA start site. Deletion of a portion of the ORF 603 from viruses containing the normal polyhedrin gene and the *lacZ* gene in lieu of polyhedrin did not affect replication in cell culture or the production of β-galactosidase protein. A virus which lacked the ORF 603 gene but produced polyhedrin had similar infectivity in *Trichoplusia ni* larvae compared to the wild-type virus. The chloramphenicol acetyltransferase (CAT) gene was also inserted in lieu of the ORF 603 in a virus containing the *lacZ* gene instead of the polyhedrin (Ac. CAT. *lacZ*). Analysis of CAT expression revealed that a maximum level was reached at 16 h p.i. and that transcription was initiated in Ac. CAT. *lacZ* at the same site as for the normal gene.

Introduction

The genome of the insect baculovirus *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus (AcMNPV) consists of approximately 130 kbp of covalently closed circular DNA (Smith & Summers, 1978; Miller & Dawes, 1979; Lübbert et al., 1981; Cochran et al., 1982). This is packaged into rod-shaped nucleocapsids which are subsequently enclosed within lipoprotein envelopes to form virus particles (see Kelly, 1985). These are further packaged within crystalline matrices consisting largely of a single 28K protein (polyhedrin) to form the polyhedra.

Virus replication is accompanied by four phases of gene expression in infected cells (Kelly & Lescott, 1981), which begin with the expression of immediate early genes in the absence of de novo viral protein synthesis. Thereafter, delayed early genes are expressed, a process dependent on the prior syntheses of the immediate early gene products and accompanied by the onset of virus DNA synthesis. The late phase of gene expression begins at about 10 h post-infection (p.i.) and is coincident with the budding of non-occluded virus particles from the plasma membrane of the cell. Finally there is a very late phase, initiated at about 15 h p.i., when copious amounts of polyhedrin and p10 proteins are produced and virus particles are occluded into polyhedra within the nucleus.

An immediate early gene (IE-1) has been described which trans-activates the expression of a delayed early/late gene (39K) (Guarino & Summers, 1986a); another immediate early gene, designated IE-N, augments this trans-activation (Carson et al., 1988). Regions of homologous DNA in the AcMNPV genome have been shown to act as transcriptional enhancers for delayed early and late gene expression (Guarino & Summers, 1986b, 1988; Guarino et al., 1986). Trans-activation of late genes has also been described (Guarino & Summers, 1988). However, as yet, little is known about the mechanisms involved in the transition from late to very late transcription. Late phases of transcription are thought to be mediated by a virus-induced α-amanitin-resistant RNA polymerase (Fuchs et al., 1983). It is possible that different forms of such an enzyme complex could have some role in phase switching. The genome organization and pattern of transcripts produced that are peculiar to AcMNPV may also be important in these processes. For example, different temporal patterns of overlapping mRNAs have been observed and sets of transcripts with common 3' termini or common 5' termini have been mapped (Lübbert & Doerfler, 1984; Friesen & Miller, 1983; Mainprize et al., 1986; Rankin et al., 1986; Rice & Miller, 1986; Oellig et al., 1987). It has
been suggested that this type of genome organization could help to co-ordinate temporal expression via interference between adjacent gene promoters (Friesen & Miller, 1985).

The polyhedrin gene promoter of AcMNPV has been studied because of its utility as an expression system to synthesize large quantities of foreign gene products in insect cells (reviewed by Luckow & Summers, 1988; Miller, 1988). The promoter has been defined as a 69 nucleotide region extending upstream from the ATG of the coding sequences (Matsura et al., 1987; Possee & Howard, 1987; Rankin et al., 1988). Friesen & Miller (1985) identified two extra transcripts of 3.39 kb and 4.9 kb, which have a 5’ end in common with that of the major polyhedrin mRNA (1.28 kb). Mainprize et al. (1986) also mapped several transcripts to a position about 1000 nucleotides upstream from the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

Methods

Viruses and cells. AcMNPV C6 (Possee, 1986) was propagated in Spodoptera frugiperda cells (IPLB-Sf-21AE) (Vaughn et al., 1977). Virus stocks were titrated as described by Brown & Faulkner (1977), except in virus-infected cells and the 5' end of this transcript extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.

In this study we sequenced a region of the AcMNPV genome extending about 2000 nucleotides upstream from the polyhedrin gene to investigate the relationship between neighbouring transcription units and polyhedrin expression. A 603 nucleotide open reading frame (ORF) was identified on the opposite strand of DNA to the polyhedrin gene using a 650 nucleotide cDNA clone as a probe. However, since the published sequence information extends only 200 nucleotides upstream from the polyhedrin coding region the fine details of transcript organization remain undefined.
AcMNPV gene analysis

![Diagram](image.png)

Fig. 1. (a) Genetic organization of the AcMNPV EcoRI fragment I. The position of the polyhedrin gene is shown, together with the sites at which Bal 31 exonuclease digestions were initiated (thick, bidirectional arrows) to produce deletions for DNA sequence analysis. The left EcoRI site is used as the zero point of the AcMNPV genome (Vlak & Smith, 1982). (b) Nucleotide sequence of the XhoI–EcoRV fragment. The sequence begins with the last three nucleotides of the XhoI site (underlined). The data also include part of the published sequence of the polyhedrin gene (nucleotides 2092 to 2400; Hooft van Iddekinge et al., 1983). The predicted protein sequence of the ORF 603 gene product is shown between nucleotides 1429 and 2031 (note that the antisense DNA sequence for this potential gene is presented). The mRNA start sites (T) for polyhedrin (2138) and ORF 603 (2135/6) are depicted.
Fig. 2. Analysis of transcription from the polyhedrin gene region. The upper panel shows the strategy for producing strand-specific radiolabelled RNA probes from pSPT vectors with T7 RNA polymerase. The EcoRV (E)–BamHI (B) fragment was inserted in pSPT19 for polyhedrin-specific RNA. A XhoI–EcoRV fragment, from a deletion mutant of pAcEI-I (see Fig. 1a), was inserted in pSPT18 to produce ORF 603-specific RNA [the new XhoI site was 43 nucleotides to the left of the MluI (M) site]. The lower left panel shows the Northern hybridization analysis of poly(A)+ RNA from uninfected S. frugiperda cells, AcMNPV-infected cells (4 to 24 h p.i.) and infected cells at 12 h p.i. treated with cycloheximide (CX). The blot was probed with the ORF 603-specific RNA transcript. The lower right panel shows a similar analysis of poly(A)+ RNA from AcMNPV-infected cells at 12 and 24 h p.i., probed with the polyhedrin-specific RNA transcript. Size markers in kb are shown.

**Results**

**Sequencing the 5' flanking region of the AcMNPV polyhedrin gene**

The AcMNPV polyhedrin gene is located within the 7.3 kbp EcoRI I DNA fragment (Vlak et al., 1981; Adang & Miller, 1982; Rohel et al., 1983; Smith et al., 1983b). This fragment is shown in Fig. 1 (a), with the position of the polyhedrin gene and the direction of transcription indicated. The plasmid pUC8/6,8, containing a modified EcoRI I fragment (Possee, 1986), was used to create a series of deletion mutants from the XhoI and EcoRV sites.
AcMNPV gene analysis 255

(a) Strategy for producing strand-specific probes. The MluI-BamHI and Sau3AI-HhaI fragments were radiolabelled (*) at the 5' ends to produce probes of 875 and 444 nucleotides, respectively. (b) The MluI-BamHI fragment was annealed to total RNA from AcMNPV-infected cells at 12 and 24 h p.i., RNA from AcMNPV-infected cells at 12 h p.i. treated with cycloheximide (CX) and yeast RNA (t). The original probe and the size of the 654/665 nucleotide protected fragment are indicated; M, size markers in nucleotides. (c) The Sau3AI-HhaI fragment was annealed to total RNA from infected cells at 12 h p.i., treated with S1 nuclease and the protected fragment was sized against a sequencing ladder from pAcRP23.

Fig. 3. S1 nuclease analysis of ORF 603 transcription. (a) Strategy for producing strand-specific probes. The MluI-BamHI and Sau3AI-HhaI fragments were radiolabelled (*) at the 5' ends to produce probes of 875 and 444 nucleotides, respectively. (b) The MluI-BamHI fragment was annealed to total RNA from AcMNPV-infected cells at 12 and 24 h p.i., RNA from AcMNPV-infected cells at 12 h p.i. treated with cycloheximide (CX) and yeast RNA (t). The original probe and the size of the 654/665 nucleotide protected fragment are indicated; M, size markers in nucleotides. (c) The Sau3AI-HhaI fragment was annealed to total RNA from infected cells at 12 h p.i., treated with S1 nuclease and the protected fragment was sized against a sequencing ladder from pAcRP23.

sites with Bal 31 exonuclease. The deletions were used to sequence both strands of DNA (Fig. 1a). These data, together with the 5' end of the polyhedrin gene (Hooft van Iddekinge et al., 1983), are shown in Fig. 1(b). Analysis of the sequence identified an open reading frame, 603 nucleotides in length (ORF 603), beginning 156 bp upstream from the polyhedrin coding sequence, in the strand of DNA opposite to the polyhedrin gene. This could potentially encode a 201 amino acid polypeptide of predicted Mr, 23568.

Transcription from ORF 603
In order to detect transcription of ORF 603, poly(A)+ RNA was extracted from AcMNPV-infected S. frugiperda cells and analysed using denaturing agarose gels
Fig. 4. Preparation of plasmids with deletions in ORF 603. The pAcEI-I clone was modified to produce pAcEI-IΔ603, which has a 240 nucleotide deletion extending from the EcoRV site to a point 177 nucleotides within the coding sequences of ORF 603. The pAcRP23 lacZΔ603 construct has an identical deletion but contains the lacZ gene in lieu of the amino terminus of polyhedrin (P). Restriction enzyme sites: E, EcoRI; X, XhoI; R, EcoRV; B, BamHI; Bg, BglII. CIP, calf intestinal phosphatase.

and Northern blot hybridization. The radiolabelled RNA probes used were specific for either ORF 603 or polyhedrin and were produced from pSPT18 or -19 vectors (see Methods and Fig. 2). The Northern blots (Fig. 2) showed that an ORF 603-specific mRNA (3.7 kb in size) was first detected at 8 h p.i., reached a peak at 12 h p.i. and then declined in level until it was undetectable by 24 h p.i. It was not observed in cycloheximide-treated AcMNPV-infected cells. In contrast, the major 1.2 kb transcript specific for the polyhedrin gene was undetectable before 12 h p.i. (data not shown) but abundant at 24 h p.i. Similar hybridization analyses used radiolabelled RNA transcripts from pSPT vectors containing the 381 bp HindIII-Asp718 fragment from the polyhedrin gene (Hoof et al., 1983) failed to detect the 3.7 kb species (data not shown).

The transcription start site of the 3.7 kb mRNA was determined using S1 nuclease protection analysis with a 5' end radiolabelled MhuI-BamHI probe of 875 nucleotides (Fig. 3a) where a fragment of approximately 654 or 655 nucleotides was protected (Fig. 3b). This was not detected when RNA from cells at 24 h p.i. was used, or if the cells had been treated with cycloheximide. The 5' end of the ORF 603 transcript was precisely mapped using a 5' end-radiolabelled Sau3A-HhaI fragment of 444 nucleotides. (Fig. 3a). This protected a fragment of 297 or 298 nucleotides, the size of which was determined against a DNA sequencing ladder (Fig. 3c). This accurately located the transcription initiation site for ORF 603 two to three nucleotides upstream from the polyhedrin mRNA start site, but on the opposite strand of DNA (Fig. 1b). The apparent size of the transcript (3.7 kb) extended beyond the DNA sequence reported in
Fig. 5. Southern hybridization analysis of virus DNA. Lane 1, AcMNPV DNA digested with XhoI and EcoRV; lanes 2 to 4, AcMNPV, AcRP23. *lacZ*Δ603 and AcEI-Δ603, respectively, digested with XhoI and BglII. The left panel is an ethidium bromide-stained 0.6% agarose gel; the right panel is a Southern blot of the gel hybridized with nick-translated pAcPCAT. M, size standards in kbp.

Functional analysis of ORF 603

The role of ORF 603 in AcMNPV replication was studied by preparing recombinant viruses lacking a functional gene; the construction of these deletion mutants was outlined in Methods and Fig. 4. The virus AcRP23. *lacZ*Δ603 had a 177 nucleotide deletion in ORF 603 and the *lacZ* gene inserted in lieu of the amino terminal polyhedrin coding sequences, although the polyhedrin promoter remained intact. The recombinant AcEI-Δ603 had the same deletion in ORF 603, but retained the polyhedrin gene.

The deletion in the ORF 603 gene of AcRP23. *lacZ*Δ603 and AcEI-Δ603 described in Fig. 4 was confirmed by Southern blot hybridization analysis of purified virus DNA (Fig. 5). AcMNPV DNA digested with XhoI and EcoRV produced the normal 2.09 kbp fragment spanning ORF 603 (lane 1). Digestion of the same DNA with XhoI and BglII highlighted a larger fragment (lane 2). However, AcRP23. *lacZ*Δ603 DNA digested with XhoI and BglII produced a 1.86 kbp fragment corresponding to the deletion of 177 bp in ORF 603 and the BglII site in lieu of the EcoRV site between

Fig. 6. Protein synthesis in cells infected with recombinant viruses. *S. frugiperda* cells (lane 1) were infected with AcRP23. *lacZ* (lanes 2 to 5), or AcRP23. *lacZ*Δ603 (lanes 6 to 9) and pulse-labelled with [14C]leucine at the following times after infection: 6 h (lanes 2 and 6), 12 h (lanes 3 and 7), 18 h (lanes 4 and 8) or 24 h p.i. (lanes 5 and 9). Protein extracts were analysed in a 12% polyacrylamide gel. B, β-galactosidase; M, standards are shown on the left of the gel.
ORF 603 and polyhedrin (lane 3). A larger fragment was also highlighted with this probe due to the simian virus 40 (SV40) sequences at the 3' end of the lacZ gene which hybridized to the homologous SV40 sequences present in the nick-translated pAcPCAT used as a probe (lane 3).

When AcEI-IA603 DNA was digested with XhoI and BglII a 1.86 kbp fragment hybridized to the probe, thus confirming the deletion in ORF 603. The SV40 sequences from the virus AcRP23.lacZΔ603 were not present, indicating that faithful recombination had taken place between the transfer vector pAcEI-IA603 and the AcRP23.lacZΔ603 virus DNA. It is also worth noting that the deletion in ORF 603 removed the normal ATG codon of this gene and that there were no alternative ATG codons in the remaining coding sequence. Furthermore, if the two ATG codons in the mRNA leader sequence (nucleotides 2121 and 2097; Fig. 1 b) were utilized by the recombinant viruses, only short peptides of 13 or 21 amino acids would be produced before translation stop codons terminated protein synthesis.

Deletion of the ORF 603 region did not affect virus replication in cell culture, as judged by the production of infectious non-occluded virus. The effect of the deletion on expression from the polyhedrin promoter was investigated by pulse-labelling cells infected with AcRP23.lacZΔ603 or AcRP23.lacZ (Possee & Howard, 1987). The cell extracts were analysed in 12% polyacrylamide gels and demonstrated that the production of β-galactosidase protein was not affected by the absence of the 240 nucleotides upstream from the EcoRV site (Fig. 6). The apparent increase in the amount of β-galactosidase protein by AcRP23.lacZΔ603 at 24 h p.i. (Fig. 6, lane 9) was not confirmed by an enzyme assay of cell extracts (data not shown). The AcRP23.lacZΔ603 recombinant did not show variation in polypeptides of 25K where the putative ORF 603 gene product would be expected to migrate (Fig. 6). Similar results were
obtained when AcMNPV and AcEI-IΔ603 (polyhedra-positive) viruses were compared (data not shown).

Biological activity of the ORF 603 gene product

Removal of most of the ORF 603 coding sequences did not affect virus replication in cell culture. However, virus replication in insect larvae might be affected, since the whole animal is a more complex environment. Therefore, the virulence of AcEI-IΔ603 was compared with that of the wild-type AcMNPV using T. ni larvae as an indicator species. Polyhedra were purified from infected S. frugiperda cells and used in a bioassay to estimate the LD50 of each virus. Insects infected with either virus died between 5 and 7 days p.i. The LD50 for each virus was calculated to be 94 polyhedra for AcEI-IΔ603 and 101 polyhedra for AcMNPV (Fig. 7).

Foreign gene expression with ORF 603 promoter

The redundancy of ORF 603 suggested that a foreign gene could be inserted in its place under the control of the ORF 603 promoter. Therefore, a CAT gene cassette was inserted in the BglII site of pAcRP23.lacZΔ603, in the appropriate orientation, to produce pAc.CAT.lacZ (Fig. 8a). The recombinant virus Ac.CAT.lacZ was derived and proved to be stable after repeated passage in cell culture. Cells infected with Ac.CAT.lacZ were harvested at different times after infection and extracts assayed for CAT activity; the results are shown in Fig. 9. Significant levels of CAT, as measured by conversion of chloramphenicol to the acetylated forms, were detected at 10 h p.i., rising to a maximum of 23.7% at 16 h p.i. Thereafter, the level of enzyme activity remained stable at least until 24 h p.i. In other experiments (data not shown) the levels of β-galactosidase produced by Ac.CAT.lacZ, AcRP23.lacZΔ603 and AcRP23.lacZ were similar, demonstrating that the polyhedrin promoter in Ac.CAT.lacZ still functioned normally.

To demonstrate that transcription from the ORF 603 promoter in Ac.CAT.lacZ was initiated at the authentic site, mRNA isolated from Ac.CAT.lacZ-infected cells was used in S1 protection experiments. Fig. 8(b, c) shows that a protected fragment of approximately 318 nucleotides was produced. This corresponded to the position of the mRNA start site determined for the genuine ORF 603 transcript (Fig. 3).

Discussion

A portion of the AcMNPV genome approximately 2 kbp upstream from the polyhedrin gene has been sequenced.
This region was found to contain a large open reading frame, 603 nucleotides in length and beginning 156 nucleotides upstream from the polyhedrin ATG codon on the opposite strand of DNA. Northern blot analysis of infected cell RNA identified a 3-7 kb polyadenylated RNA transcribed from this region. This RNA was maximally synthesized at 12 h p.i., but was not detected in infected cells at 24 h p.i. Production of this transcript was dependent on the synthesis of earlier virus gene products since it was undetectable in cycloheximide-treated cells. The transcript could correspond to an mRNA species mapped to this region by Mainprize et al. (1986), which was present in infected cells at 12 h p.i., although apparently maximally transcribed at 24 h p.i.

The RNA start site was preliminarily mapped to a position just two or three nucleotides upstream of the polyhedrin mRNA start site (Howard et al., 1986) and thus suggests that the polyhedrin promoter acts in a bidirectional fashion.

Bidirectional transcription in AcMNPV-infected cells has been described previously where a single long terminal repeat of a retrotransposon (TED) inserted in the genome of the virus was found to direct divergent transcription from a single point (Friesen et al., 1986). The TED RNAs initiated within a region of partial dyad symmetry. In contrast to this the polyhedrin promoter is not symmetrical. The divergent transcripts from polyhedrin and ORF 603 were produced at different times in infection; ORF 603 transcripts were observed between 8 and 18 h p.i. with a peak at 12 h p.i., coinciding with the activation of transcription of the very late polyhedrin gene, but were not detectable at 24 h p.i. Transcripts from ORF 603 decreased in abundance as the polyhedrin mRNA accumulated. These data suggest that ORF 603 transcription was suppressed as a consequence of activation of polyhedrin transcription. Alternatively, transcription from ORF 603 may depend on protein factors or an RNA polymerase which are depleted late in infection. There is evidence that a novel α-amanitin-resistant RNA polymerase is produced in AcMNPV-infected cells from about 6 h p.i. and that most of the late viral transcription is resistant to this drug (Fuchs et al., 1983). This enzyme may displace other polymerases from the promoter region and thus afford transcription of polyhedrin instead of ORF 603. Although our data suggest that the polyhedrin promoter may act to direct transcription in both directions, albeit at different times after infection, caution must be exercised here. We have only used nuclease S1 analysis to map the 5' end of the ORF 603-specific transcript and it would be interesting to see whether primer extension analysis gives the same result. Furthermore, the authenticity of our data should be confirmed by using site-directed mutagenesis to modify the 12 nucleotide consensus sequence spanning the transcription start site and thus determining whether synthesis of either transcript is affected. These experiments are in progress.

Virus deletion mutants lacking the 5' end of the ORF 603 still produced normal quantities of polyhedrin or β-galactosidase proteins at the expected time after infection. Deletion of part of ORF 603 did not affect virus replication in cell culture and the virus titres attained by the deletion mutants matched those of the normal virus. When the infectivity of polyhedra for insect larvae was compared, the LD₅₀ values for each virus were very similar. These data suggest that the putative gene product for ORF 603 is not essential for virus replication. However, it is conceivable that the protein may have a role in tissue tropism within the insect larvae. We were unable to observe any difference in intracellular polypeptides produced by the recombinant virus compared with normal AcMNPV; therefore it is not possible to conclude that a protein is synthesized in infected cells. Future studies will have to establish whether a polypeptide is produced by this gene and where it is localized within the infected cells and insect.

It is planned to express ORF 603 in a heterologous system, produce antiserum and use this to probe protein blots of infected cells to identify the gene product. However, the virus deletion mutants lacking a functional ORF 603 did retain a portion of DNA extending 41 nucleotides upstream from the ORF 603 RNA start site. To determine whether this was sufficient to direct the synthesis of RNA the deletions made in ORF 603 were replaced with the CAT gene. This recombinant virus (Ac. CAT. lacZ) was able to synthesize significant amounts of CAT enzyme at the times expected for ORF 603 promoter activity. Furthermore, the polyhedrin promoter remained active, as evidenced by the levels of β-galactosidase produced. The transcript produced by the ORF 603 promoter was initiated at the expected mRNA start site.

A further consequence of these data is that more information is now available on the sequence requirements for polyhedrin expression. It has been suggested that sequences characteristic of eukaryotic promoter elements in the 5' flanking region of the polyhedrin gene may be important in the regulation of transcription (Hooft van Iddekinge et al., 1983). A TATA-like element located 24 to 33 nucleotides from the polyhedrin transcription start site was previously shown not to be necessary for polyhedrin transcription (Possee & Howard, 1987). A CAAT-like sequence (TATCAATAT) between nucleotides 2074 and 2082 and a pair of tandem repeats (CACAACT) between nucleotides 2044 to 2051 and 2057 to 2064 were also identified (Fig. 2; Hooft van Iddekinge et al., 1983). Here we have shown that removal of these sequences did not affect expression
from the polyhedrin promoter in the recombinant viruses AcE1-IA603 or AcRP23, lacZΔ603. This confirms that only 69 nucleotides upstream from the polyhedrin gene are apparently required for efficient promoter activity (Matsuura et al., 1987; Possee & Howard, 1987). It remains to be determined how much of this intergenic region is required to promote ORF 603-specific transcription and whether the proximity of these neighbouring transcription units to each other has any functional role in the temporal regulation of baculovirus gene expression.

We thank Chris Hatton for photographic services. Katy Gearing was supported by a NERC Research Studentship.

References


(Received 20 April 1989; Accepted 17 October 1989)