Characterization of the DA26 gene in a hypervariable region of the Autographa californica nuclear polyhedrosis virus genome

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A region of the baculovirus Autographa californica nuclear polyhedrosis virus genome that is frequently found to be altered after serial passage of the virus in cell culture was characterized. Sequence analysis of this region of the genome in wild-type and mutant viruses revealed that some of the mutations affected a 675 bp open reading frame, designated DA26. The DA26 gene was disrupted both by deletion and by insertion of sequences that resembled transposable elements. Northern blot analysis of DA26 showed that it was expressed very early after infection. DA26-specific transcripts could be detected after the 1 h viral adsorption period upon infection of cultured Trichoplusia ni cells. These transcripts were mapped by nuclease protection assays. A recombinant virus was constructed in which DA26 was disrupted by insertion of the Escherichia coli lacZ gene. This virus was viable in both T. ni and Spodoptera frugiperda cells and analysis of the kinetics of protein synthesis revealed no differences between wild-type and recombinant viruses. The disruption of DA26 also did not interfere with the ability of the virus to infect T. ni or S. frugiperda larvae.

Introduction

Serial passage of Autographa californica nuclear polyhedrosis virus (AcMNPV) in cell culture gives rise to mutant viruses that often become the predominant species in viral stocks, presumably because the mutations confer some selective advantage for growth in cell culture (Potter & Miller, 1980; Burand & Summers, 1987). A selective advantage has been demonstrated for viruses displaying the ‘few polyhedra’ (FP) phenotype. Such mutants form plaques with very few polyhedral occlusion bodies per infected cell (Hink & Vail, 1973; Potter et al., 1976; Fraser & Hink, 1982). Potter et al. (1976) have shown that FP mutants produce a higher titre of budded virus than wild-type (wt) virus, apparently at the expense of occluded virus production. The FP phenotype has been correlated with insertions in the HindIII I region of the AcMNPV genome by marker rescue (Fraser et al., 1983). A gene encoding a 25K protein is disrupted by these insertions (Fraser et al., 1983; Beames & Summers, 1988, 1989; Wang et al., 1989). The insertions seem to be host transposable elements, which are derived from, and often specific to, the host cell in which the virus was passaged (Miller & Miller, 1982; Fraser et al., 1983; Carstens, 1987; Beames & Summers, 1988; Cary et al., 1989; Wang et al., 1989).

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There is evidence that mutations in other regions of the viral genome can also confer a selective advantage to the virus for growth in cell culture. A comprehensive study in which viruses were isolated, without phenotypic selection, from stocks of AcMNPV serially passaged through Trichoplusia ni cells showed that three different classes of mutations predominated (Kumar & Miller, 1987). These mutations were deletions in the PstI G region (7.6 to 13.1 map units (m.u.); discussed above). We have been studying the nature of the mutations occurring within the PstI G region of the genome. The data of Kumar & Miller (1987) showed that approximately half of the 667 viruses derived from 24 independently passaged stocks had incurred a deletion of about 1 kb around a PvuII site at 9.5 m.u. We have shown that these viruses have sustained identical deletions of 1065 bp within the egt gene of AcMNPV (O'Reilly & Miller, 1990). The egt gene encodes an ecdysteroid UDP-glucosyl transferase, which enables the virus to control the moulting and pupation of its insect host (O'Reilly & Miller, 1989).

In the course of our studies on mutations within PstI G, we noted that the right breakpoint of the 1065 bp deletions lies very close to a neighbouring open reading frame (ORF), DA26, and that the deletions would disrupt transcription of this gene. DA26 has previously been implicated in the regulation of late gene expression...
by transient expression assays (Guarino & Summers, 1988). In this study we show that two insertional mutations from serially passaged virus mutants map within DA26. In addition we show that two deletions arising from serial passage of AcMNPV through Manduca sexta (tobacco hornworm) disrupt DA26. We demonstrate that the DA26 gene is an early gene that is not essential for viral replication, either in tissue culture or in insect larvae. It does not appear to play a general role in the regulation of late gene expression.

**Methods**

**Cells and viruses.** *T. ni* (cabbage looper) TN368 (Hink, 1970) and *S. frugiperda* (fall armyworm) IPLB SF21 (Vaughn et al., 1977) cells were maintained in TC100 medium (Gibco) supplemented with 10% foetal calf serum (Gibco) and 0.25% tryptose broth. Wild-type AcMNPV L-1 (Lee & Miller, 1978) and mutant viruses were propagated and titrated on SF21 cells as described (Miller et al., 1986), unless otherwise stated. Mutant viruses vMSL A and vMSLB were isolated after serial passage of AcMNPV through *M. sexta* larvae (D. J. Browne & L. K. Miller, unpublished results). The insertional mutations v4c19 and v5a25 were identified following serial passage through TN368 cells (Kumar & Miller, 1987).

**Cloning and sequencing.** The procedures used for the cloning and sequencing of the wt AcMNPV genome, from 8-65 to 10.3 m.u., have been described elsewhere (O'Reilly & Miller, 1990). The analogous regions of the mutants vMSL A and vMSLB, v4c19 and v5a25 were cloned and sequenced in a similar manner.

**Northern blot analysis of DA26 transcripts.** Since the mutants v4c19 and v5a25 were isolated following serial passage through TN368 cells, DA26 transcription was examined in both SF21 and TN368 cells to investigate whether expression of the DA26 gene product was similar in both cell types. SF21 or TN368 cells were infected with AcMNPV at an m.o.i. of 20 and total RNA was isolated at various times post-infection (p.i.) by guanidinium isothiocyanate lysis and pelleting the 5' and 3' ends of DA26 transcripts. SF21 or TN368 cells were infected with AcMNPV at an m.o.i. of 20 and total RNA was isolated at various times post-infection (p.i.) by guanidinium isothiocyanate lysis and pelleting.

**Mapping of DA26 transcripts.** The 5' and 3' ends of DA26 transcripts were mapped by S1 nuclease protection assays using end-labelled probes as described previously (O'Reilly & Miller, 1990).

**Construction of the vDA26Z recombinant virus.** The plasmid pBSBCES, which includes AcMNPV sequences from the EcoRI site at 8-65 m.u. to the SalI site at 10.5 m.u., was cleaved at the BstXI site within DA26 at nucleotide (nt) 2169. The *E. coli lacZ* gene was excised from the plasmid pSK5105 (Casadaban et al., 1983) by cleavage with *SalI*. After end-filling this fragment was inserted into pBSBCES, yielding a plasmid, pDA26Z, with the *lacZ* gene inserted in-frame following amino acid 118 of ORF DA26. The *lacZ* gene is flanked on either side by 1-7 and 0-6 kb of viral sequences. These allowed allelic replacement of the DA26-*lacZ* fusion into the viral genome following contransfection of pDA26Z and wt viral DNA. The recombinant virus, vDA26Z, was selected by screening for *β*-galactosidase expression (Penneck et al., 1984) and characterized by restriction enzyme and Southern blot analysis.

**Analysis of protein expression.** SF21 or TN368 cells were infected with wt AcMNPV or the recombinant vDA26Z for various periods of time and pulse-labelled with 25 μCi [35S]methionine (New England Nuclear) for 1 h before lysis as described (O'Reilly & Miller, 1988). Lysates were analysed by SDS-PAGE and fluorography.

**Insect bioassays.** Neonate Spodoptera frugiperda and *T. ni* larvae were infected by feeding with artificial diet (Burton, 1969; Treat & Halfhill, 1973) containing various concentrations of wt AcMNPV or vDA26Z polyhedra. The doses used for infection of *T. ni* larvae were 2000, 4000, 8000, 16000, 32000 and 64000 polyhedra/ml, whereas doses of 150000, 300000, 600000, 1200000, 2400000 and 4800000 polyhedra/ml were used for *S. frugiperda*. Larvae were allowed to feed on the contaminated diet for 24 h and then transferred to virus-free diet. Seventy-five larvae were infected with each dose. Polyhedra used in each determination were all prepared by infection of the same batch of *T. ni* larvae, as described (Miller & Dawes, 1978). Mortality was recorded 7 days after infection of *T. ni* and 10 days after infection of *S. frugiperda* and the data obtained were analysed by probit analysis (Daum, 1970).

**Results**

**Nucleotide sequence analysis of wt virus**

We have determined the nucleotide sequence of a *SalI* fragment of the AcMNPV genome, extending from 8-3 to 10.5 m.u. Residues 1 to 1800 of this sequence encompass the egt gene and are presented in another report (O'Reilly & Miller, 1990), whereas residues 1600 to 2793 are presented here. The sequencing strategy used and the sequence obtained are presented in Fig. 1. Two ORFs were identified in the sequence (Fig. 1c and d). The larger of these consists of 225 codons and has the potential to encode a very basic protein (pI = 11.44) of *M.* 25890. The sequence of the smaller ORF does not extend to its C terminus. However, this region of AcMNPV has also been sequenced by Guarino & Summers (1988) and, based on their sequence data, this gene would be expected to extend an additional 53 codons. It could therefore specify a slightly acidic protein (pI = 5-92) of *M.* 18400. There are no discrepancies between the nucleotide sequence we present here and the sequence data of Guarino & Summers (1988). We have designated these genes DA26 (for *HindIII* D, EcoRI A, 26K) and DA18, respectively. Neither gene displays significant homology to any sequence present in GenBank, release 55.

**Nucleotide sequence analysis of virus mutants**

Nucleotide sequence analysis of v1a7, which is typical of the large number of deletions observed within *PstI* G
AcMNPV DA26 gene expression and function

Fig. 1. Sequencing strategy and sequence of the AcMNPV DA26 gene. The sequencing strategy used and a map of the region sequenced are presented in (a) and (b). The coordinates of the region, in m.u., are given below (b). (c) Occurrence of stop codons (depicted by vertical lines) in all six reading frames. The nucleotide sequence and predicted amino acid sequence are presented in (d). The + symbol denotes the position of the 5' end of the 1-7 kb early transcript, whereas the asterisks above the sequence mark the late consensus initiation site ATAAG. Double underlined sequences represent potential transcription control signals (TATA box, GTGT motif). The endpoints of the deletions in vla7, vMSLA and vMSLB are indicated (the arrows show the terminal residues deleted). The duplicated viral sequences that flank the insertions in v4c19 and v5a25 are in bold and underlined and the long arrows above the sequence represent the imperfect palindrome around the v5a25 insertion site.

following serial passage of the virus in T. ni cells (Kumar & Miller, 1987), had revealed that the deletion extends from within the egt gene to nt 1779, 33 bp upstream of the DA26 translational start site (O'Reilly & Miller, 1990; see Fig. 1d). Thus, the vla7 deletion does not disrupt the DA26 coding sequences. However, restriction enzyme analysis indicated that the mutants v4c19 and v5a25 had incurred large insertions in this region of the PstI G fragment (Kumar & Miller, 1987). Sequence analysis reveals that, in both cases, the insertional event has occurred within the DA26 gene (Fig. 1d, 4). Both insertion elements display features characteristic of transposable elements; they include short inverted repeats at each end and viral sequences are duplicated at the point of insertion (see Fig. 1d). v4c19 contains an insertion of 1530 bp flanked by a duplication of nt 2268 to 2275 (ACATTGTA), whereas the insertion element in v5a25 is 780 bp long and nt 2439 to 2442 (TTAA) are repeated (Fig. 1d).

Restriction enzyme analysis also suggested that the mutants vMSLA and vMSLB, isolated following serial passage through M. sexta larvae, had incurred deletions that extended into this region (D. J. Browne & L. K. Miller, unpublished observations). Sequence analysis of these mutants reveals that the DA26 gene is disrupted in both cases (Fig. 1d, 4). The deletion in vMSLA extends from nt 829, within egt, to nt 2116, whereas the vMSLB deletion begins upstream of egt, at nt 52 and extends to nt 2192. Therefore, the right breakpoints of both these mutations lie within the DA26 gene.
Northern blot analysis of DA26 transcription

In order to characterize the transcriptional regulation of DA26 we first employed Northern blot analysis. SF21 and TN368 cells were infected with wt AcMNPV L-1 at an m.o.i. of 20 and total RNA was isolated at various times p.i. The dependence of DA26 transcription on prior viral protein synthesis or DNA replication was evaluated using the protein synthesis inhibitor cycloheximide and the DNA synthesis inhibitor aphidicolin. The strand-specific probe used is depicted in Fig. 2 (e). Early after infection two major transcripts, of estimated sizes 3.0 and 1.7 kb, were observed in both TN368 (Fig. 2a) and SF21 (Fig. 2b) cells. (Note that RNA for the 0 h samples was isolated at the end of the 1 h viral adsorption period.) Both these RNAs were produced in the presence of cycloheximide (Fig. 2a, b, lane 12C), indicating they are early transcripts. Later in infection a third 1.4 kb transcript was detected. This RNA was a ‘late-class’ RNA in both cell lines as aphidicolin and cycloheximide prevented its expression. When a similar blot with RNA from infected SF21 cells was probed with a DA18-specific probe the same pattern was observed (data not shown), suggesting that the transcripts observed extend through DA18 as well.

The overall pattern of transcription from this region was qualitatively similar in SF21 and TN368 cells (compare Fig. 2a and b). However, some quantitative differences were observed; the relative abundance of transcripts was different and early transcripts, in particular the 1.7 kb RNA, appeared earlier after infection of TN368 cells (compare 0 and 3 h lanes, Fig. 2a and b).

Mapping the 5' ends of DA26 transcripts

Nuclease protection assays were used to locate the termini of the DA26-specific transcripts. The probe used to map the 5' termini was end-labelled at a NruI site within DA26 (Fig. 2e). Three major protected frag-
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Fig. 3. 3' End-mapping of DA26 transcripts. Total RNA isolated at various times after infection of TN368 (a) or SF21 (b and c) cells with wt AcMNPV was annealed with a 3' end-labelled probe (see d) and treated with S1 nuclease. Probe fragments protected by nuclease digestion were visualized by autoradiography of denaturing polyacrylamide gels. (d) The two probes used, the major transcripts and the resulting protected fragments; (a) and (b) display the results of nuclease protection experiments using probe A, whereas the data presented in (c) were obtained using probe B. All symbols and lane designations are as described in the legend to Fig. 2.

ments, of sizes 870, 590 and 340 nt, were observed (Fig. 2a and b). The 870 nt fragment corresponds to protection of all the viral sequences in the probe and indicates a transcript initiating upstream of the region studied here. This was expected since the transcriptional analysis of the egt gene indicated that the 3.0 kb transcript extended through this region (see O'Reilly & Miller, 1990). The 590 nt protected fragment suggested the existence of an early start site 30 to 40 nt upstream of the DA26 ATG, whereas the 340 nt fragment indicated the occurrence of a late start site within DA26.

The 5' end indicated by the 590 nt fragment was precisely located by primer extension analysis of TN368 RNA isolated 6 h p.i. These experiments demonstrated the existence of a start site 36 nt upstream of the DA26 ATG (data not shown). It is interesting to note that the sequence motif ATAAG occurs from nt 2029 to 2033, 33 to 36 nt upstream from the end-labelled NruI site. This element has been found at the start site of all late transcripts identified so far (Thiem & Miller, 1989 and references therein) and so it is likely that the 340 nt protected fragment (Fig. 2c) represents a late transcript also initiating from this motif. Guarino & Summers (1988) have also identified an early start site upstream of DA26 (they mapped this site 2 nt downstream of the site identified here) and a late start site in the vicinity of the ATAAG motif. The temporal regulation of these start sites indicated that they correspond to the early 1.7 kb RNA and the late 1.4 kb RNA, respectively. There was generally good agreement between the patterns observed in the Northern blot analyses (Fig. 2a, b) and the results obtained in these experiments. One discrepancy was the detection by nuclease protection of the 1.4 kb late transcript in the presence of aphidicolin in TN368 cells (340 nt fragment, lane 12A, Fig. 2c).

Mapping the 3' ends of DA26 transcripts

Our initial experiments to locate the 3' ends of DA26-specific transcripts used a probe 3' end-labelled at the BstXI site within DA26 and extending to the SalI site in DA18 (probe A, Fig. 3d). Nuclease protection experiments using this probe and RNA derived from infected SF21 or TN368 cells revealed a major protection product of 620 nt (Fig. 3a, b). This corresponded to protection of all the viral sequences in the probe and indicated that the transcripts extended past the SalI site. We therefore constructed a second probe, which was 3' end-labelled at the NruI site in DA26 and included viral sequences as far as the BamHI site at 11.1 m.u. (probe B, Fig. 3d). Use of this probe allowed the detection of several protected fragments, suggesting these transcripts have hetero-
genuine 3' termini. The major fragments observed were 490 and 800 nt (Fig. 3d), corresponding to transcription termini approximately 60 and 360 nt downstream of the SalI site, respectively. A 3' end 60 nt downstream of the SalI site would lie within DA18.

The sequence data and results of transcriptional analyses reported in this manuscript and in O’Reilly & Miller (1990) are summarized in Fig. 4. It should be emphasized that only major transcripts are depicted here. The nuclease protection experiments do not reveal which transcripts terminate at which sites. However, based on the estimated sizes of the transcripts, it is likely that the 3-0 kb RNA terminates within DA18, whereas the 1-7 kb and 1-4 kb transcripts extend beyond it.

**Phenotype of DA26 mutant in cell culture**

The observation that DA26 is disrupted in many viral mutants with alterations in this region indicated that the DA26 gene product is non-essential for viral replication. However, it was likely that the mutants examined also had alterations elsewhere in their genome, making it more difficult to assign a phenotype to DA26 mutants. Therefore, we constructed a recombinant virus (vDA26Z) with the E. coli lacZ gene inserted into DA26 at the BstXI site (Fig. 4). This mutant is otherwise identical to wt AcMNPV. SF21 and TN368 cells were infected with vDA26Z or wt AcMNPV at an m.o.i. of 20 and at the appropriate times after infection (see Fig. 5), they were pulse-labelled with [35S]cysteine for 1 h and lysed. Whole cell lysates were fractionated through 12% SDS–polyacrylamide gels and visualized by fluorography (Fig. 5). The most notable feature of the data obtained in this experiment is that the infection was found to proceed with the same kinetics with either virus (compare Fig. 5a with b, and c with d). The temporal regulation of virus-specific proteins and the shut-off of host cell protein synthesis are similar in each case. Note in particular that there is no detectable delay or deficiency in late viral gene expression in cells infected by vDA26Z. The only apparent difference between wt- or mutant virus-infected cells is in the levels of a 30K protein expressed at 4 and 6 h p.i. The rates of synthesis of this protein appear to be elevated following vDA26Z infection of either cell line and whereas this protein may be absent from wt-
Fig. 5. Kinetics of protein expression in wt AcMNPV- and vDA26Z-infected cells. TN368 (a and b) and SF21 (c and d) cells were infected with wt virus (a and c) or recombinant vDA26Z (b and d). For infection of TN368 cells, virus stocks were assayed on TN368 cells; stocks for infection of SF21 cells were titrated as usual on SF21 cells. At selected times p.i. the cells were pulse-labelled with $^{[35]S}$cysteine for 1 h before lysis. The times of lysis are indicated in h above each panel. Labelled proteins were visualized by SDS-PAGE and fluorography. The sizes of the $M_r$ markers are shown.

Table 1. Biological activity of wt AcMNPV and vDA26Z in first instar T. ni and S. frugiperda larvae

<table>
<thead>
<tr>
<th>Host</th>
<th>Virus</th>
<th>LC$_{50}$ (polyhedra/ml)</th>
<th>95% Fiducial limits</th>
<th>Chi$^2$ (degrees of freedom)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>Higher</td>
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<td>T. ni</td>
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<td>$1.90 \times 10^4$</td>
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<tr>
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<td>$1.76 \times 10^6$</td>
<td>$2.82 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>vDA26Z</td>
<td>$9.62 \times 10^5$</td>
<td>$7.85 \times 10^5$</td>
<td>$1.24 \times 10^6$</td>
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<tr>
<td>S. frugiperda</td>
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<td>$1.76 \times 10^6$</td>
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<td>vDA26Z</td>
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infected TN368 cells, it appears to be present at reduced levels (relative to vDA26Z infection) in wt-infected SF21 cells.

We were not able to detect clearly any products of the size expected for the DA26 gene product or the DA26–lacZ fusion product. However, $\beta$-galactosidase activity was detected after infection of either SF21 or TN368 cells with vDA26Z, indicating that the DA26–lacZ fusion product is expressed in these cells (unpublished results).

We have also found that vDA26Z infection of either SF21 or TN368 cells yields the same number of progeny occlusion bodies per cell as wt infection (data not shown), which indicates that vDA26Z does not display an FP phenotype.

Bioassay of vDA26Z

The experiments described above indicated that disruption of DA26 does not adversely affect the ability of the virus to grow in cell culture. To examine a possible role for DA26 in infection of the insect host, bioassays were carried out with wt and mutant virus on both S. frugiperda and T. ni larvae. Selected concentrations of vDA26Z or wt AcMNPV polyhedra were fed to neonate S. frugiperda or T. ni larvae and larval mortality was recorded 10 and 7 days later, respectively. The results of these experiments are presented in Table 1. In both species the LC$_{50}$ of vDA26Z is two- to three-fold lower than that of the wt virus. Thus, the inactivation of DA26 by lacZ insertion appears to increase the infectivity of
the virus somewhat. We are unsure of the basis for this effect, but it is clear that DA26 is not essential for virus replication in the infected larvae. Furthermore, visual examination of larvae infected with wt or recombinant viruses revealed no striking differences between them.

**Discussion**

This report describes the characterization of an early gene of AcMNPV that is non-essential for virus growth in cell culture or in insect larvae. The region of the AcMNPV genome containing this gene was frequently found to be mutated upon serial passage of the virus in cell culture or through *M. sexta* larvae (Kumar & Miller, 1987). Sequence analysis of wt and mutant viruses revealed that of the three ORFs (*egt*, DA26 and DA18) in this region, only *egt* and DA26 were disrupted by these mutations (see also O'Reilly & Miller, 1990). However, several of the alterations would be expected to disrupt DA18 transcription and it is possible that expression of this gene is affected in these mutants. Although the deletion in *v*a7-like mutants does not disrupt the DA26 coding sequence, sequences upstream from and including the initiation site of the 1-7 kb DA26 transcript are deleted (Fig. 1 and Fig. 4). In this class of mutant DA26 might still be transcribed from the 3-0 kb mRNA initiation site upstream of *egt* (Fig. 4).

The 1-7 kb DA26 mRNA is regulated as an early transcript in both SF21 and TN368 cells. This transcript is produced extremely rapidly during infection of TN368 cells and accumulates to high levels by the end of the 1 h viral adsorption period. The DNA sequence upstream of the 1-7 kb start site includes a TATA box-like element (29 to 25 nt upstream of the start site) and the sequence ATCTGTGT(TT) (152 to 143 nt upstream), which is similar to the sequence motif a/ctcGTGTn(ct)/ found upstream of other baculovirus early genes (Tomalski et al., 1988; Nissen & Friesen, 1989).

Many of the mutant viruses isolated after serial passage in cell culture that had alterations in the *PstI* G region, including *v*a19 and *v*a25, have been found to display an FP phenotype (Kumar & Miller, 1987). However, those results do not necessarily demonstrate that the *PstI* G fragment is implicated in the development of an FP phenotype, since all the variant viruses examined might also have mutations elsewhere in their genome. The data obtained in this study from the analysis of the recombinant virus vDA26Z show that disruption of the DA26 gene alone does not give rise to an FP phenotype.

The mutagenesis of baculoviruses by insertion of transposon-like elements has been observed previously (Miller & Miller, 1982; Fraser et al., 1983; Carstens, 1987; Beames & Summers, 1988). Although the insertion element in *v*a19 does not appear to be related to any previously described elements, the element found in *v*a25 is very similar to the TFP3 elements described previously (Fraser et al., 1983; Wang et al., 1989). TFP3 elements, which are derived from *T. ni* cells, are from 780 to 830 nt long, contain an internal *PstI* site and duplicate the sequence TTAA upon insertion (Fraser et al., 1985; Wang et al., 1989). The motif TTAA is duplicated at the site of insertion of many insertion elements found in baculoviruses (Carstens, 1987; Beames & Summers, 1988; Cary et al., 1989; Wang et al., 1989). However, it seems clear that additional sequences contribute to the specificity of insertion. It has been noted that the TTAA motif at insertion sites is often part of a palindromic sequence and frequently lies within an A+T-rich region (Cary et al., 1989; Wang et al., 1989). We note that the sequences around the TTAA insertion site in *v*a25 are also A+T-rich and contain a 14 bp imperfect palindrome (see Fig. 1d). No features of this type are observed around the *v*a19 insertion site.

DA26 corresponds to the ORF 1 previously described by Guarino & Summers (1988), who proposed that DA26 (ORF 1), in cooperation with another gene, is involved in the activation of baculovirus late gene expression. These data were obtained by monitoring transient expression of a reporter gene, chloramphenicol acetyltransferase, linked to a late gene promoter, with or without cotransfection of DA26 and the other activator gene. The authors note that the stimulation observed was not due to an increase in the steady-state levels of mRNA, suggesting that the effect observed was post-transcriptional. The *in vivo* data we present here show that DA26 is dispensable for viral growth both in cell culture and in the insect host. Examination of the kinetics of protein synthesis following infection of SF21 or TN368 cells with vDA26Z revealed no signs of a deficiency or delay in late gene expression. Thus, we have not been able to confirm a general role for DA26 in the control of late gene expression after viral infection *in vivo*. However, we have not excluded the possibility that DA26 contributes to the expression of certain specific late gene(s).

It is interesting that the disruption of DA26 appears to increase the infectivity of the virus. This observation makes it more difficult to understand why the virus carries and expresses this gene. It should be noted that we have not ruled out the possibility that the increased infectivity is due to the expression of β-galactosidase rather than the disruption of DA26. Nonetheless, it is clear that DA26 is not essential for viral replication, either in cell culture or in the insect. It is possible that DA26 is only required in very specific circumstances (e.g. infection of a specific host or tissue), or that it facilitates viral replication in some way not revealed by our *in vivo* bioassays.
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