Journal of General Virology (2001), **82**, 299–305. Printed in Great Britain

P34.8 (GP37) is not essential for baculovirus replication

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Previous reports have indicated that *p34.8* (*gp37*) may be essential for the replication of *Autographa californica* nucleopolyhedrovirus (AcMNPV) because no virus with inactivated *p34.8* was isolated. We have ascertained the requirement for this gene by attempting to inactivate it with a large insertion [the gene encoding GFP (green fluorescent protein)] or by deleting all the conserved domains from the open reading frame (ORF). The gene encoding GFP was inserted into the *Not*I site of the *p34.8* ORF and a viral plaque containing the insertion was propagated in SF-21 cells. Similarly, 531 bp (*Not*I–*Xba*I) containing all conserved domains were deleted from the ORF. All mutants were authenticated by PCR amplification, restriction endonuclease analysis, DNA sequencing, and Southern and Northern blot analysis. It was found that inactivation of *p34.8* of AcUW1-LacZ (AcMNPV containing a *lacZ* gene in the *p10* locus) had no effect on the biological property of virus, such as virulence and kinetics. These two independent methods showed that *p34.8* is not essential for replication and that this locus could provide another site for the engineering of baculoviruses.

Introduction

The baculovirus genome contains about 150 genes and open reading frames (ORF) some of which are referred to as auxiliary genes that are not essential for replication but give the virus a certain selective advantage in nature (O'Reilly, 1997). The virus also contains essential genes that are required for replication and cannot be deleted from the genome. The *p34.8* gene (homologous to *gp*37 and spindlin) of *Autographa californica* nucleopolyhedrovirus (AcMNPV) was thought to be essential because a *p34.8*-null mutant could not be isolated in cell lines (Wu & Miller, 1989). Apart from this report, other attempts made to examine the requirement of *p34.8* or its homologues for virus replication have not been published. Baculovirus *p34.8* homologues that have been sequenced share 30–40% amino acid identity with entomopoxvirus fusolins. Both proteins contain five highly conserved domains which are presumably involved in function (Gross *et al.*, 1993; Liu & Carstens, 1996; Phanis *et al.*, 1999; Li *et al.*, 2000). Fusolin is not essential for EPV replication and appears to enhance the infectivity of baculoviruses (Gross *et al.*, 1993; Liu & Carstens, 1996; Dall *et al.*, 1993; Gauthier *et al.*, 1995; Hayakawa *et al.*, 1996; Mitsuhashi *et al.*, 1997, 1998; Afonso *et al.*, 1999). Recently, the homologue of *p34.8* and its encoded product (termed spindlin) in *Choristoneura fumiferana* defective nucleopolyhedrovirus (CfDEFNPV) were characterized (Li *et al.*, 2000). Spindlin was shown to accumulate as bipyramidal crystals in the cytoplasm of infected cells. The function of P34.8 is still a matter of inference to fusolin and is yet to be elucidated. Since the two homologues are so closely related, the question arose whether the gene encoding fusolin could substitute *p34.8* in AcMNPV. Therefore, the essentiallity of *p34.8* first had to be confirmed. It has also been reported that P34.8 is associated with the occlusion bodies (OBs) of AcMNPV (Gross *et al.*, 1993). If this protein has a similar enhancing function as fusolin, then inactivation of *p34.8* may lead to a reduced potency of the virus. In this paper, we report experiments designed to inactivate *p34.8* and determine the effects on LD_{50} and virus replication kinetics.

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Methods

■ **Construction of transfer vectors to inactivate *p34.8*.** The virus used in these experiments was AcUW1-LacZ, which is an AcMNPV carrying a *lacZ* gene in the *p10* locus (Pharmingen). The green fluorescent protein (GFP) ORF was excised from pEGFP-1 (Clontech) by digesting with *Bam*HI and *Not*I (*Not*I site was blunted with Klenow). The fragment was purified on agarose gels and cloned into the *Bam*HI and *Xho*I (*Xho*I

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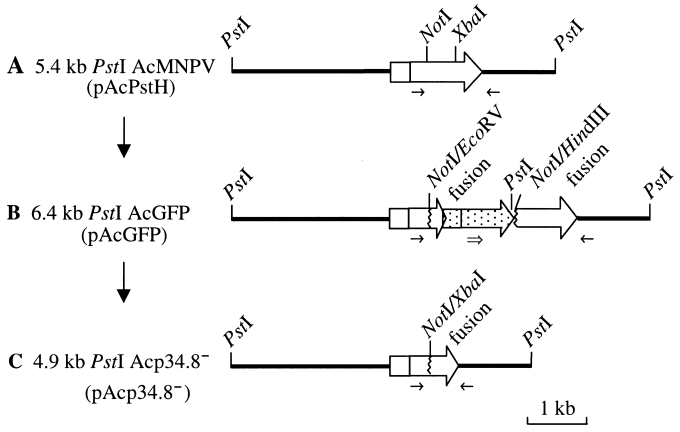

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1     MIALLIALFAAIHAPAVRSHGYLSVPTARQ      P34.8
1     MIALLIALFAAIHAPAVRSHGYLSVPTARQ      P34.8⁻

31    YKCFKDGNFYWPDNGDNIPDAACRNAYKSV      P34.8
31    YKCFKDGNFYWPDNGDNIPDAACRNAYKSV      P34.8⁻

61    YYKYRALDLESGAAASTAQYMFQQYMEYAA      P34.8
61    YYKYRALDLESGAA----------------      P34.8⁻

91    VAGPNYDDFDLIKQRVVPHTLCGAGSNDRN      P34.8
75    -------------------------------     P34.8⁻
          I                 II

121   SVFGDKSGMDEPFNNWRPNTLYLNRYQPVY      P34.8
75    -------------------------------     P34.8⁻
            III

151   QMNVHFCPTAIHEPSYFEVFITKSNWDRRN      P34.8
75    -------------------------------     P34.8⁻
                    IV

181   PITWNELEYIGGNDSNLIPNPGDSLCDNSL      P34.8
75    -------------------------------     P34.8⁻

211   VYSIPVVIPYRSNQFVMYVRWQRIDPVGEG      P34.8
75    -------------------------------     P34.8⁻
                              V

241   FYNCADLVFETLDDECRYAQMAKVVRSQLQ      P34.8
75    -----------LDDECRYAQMAKVVRSQLQ      P34.8⁻

271   KHKLDARIDHNDEESCWRARKSNYSSFFNP      P34.8
94    KHKLDARIDHNDEESCWRARKSNYSSFFNP      P34.8⁻

301   GF                                  P34.8
124   GF                                  P34.8⁻
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Fig. 1. Manipulation of the *p34.8* locus. (A) The AcUW1-LacZ 5·4 kb *Pst*I-H fragment containing *p34.8* (B) Insertion of the gene encoding GFP into the *Not*I site of *p34.8*. (C) Deletion of 531 bp including the conserved domains from *p34.8*. Transfer vectors (in parentheses) are *Pst*I fragments in pUC18. The arrows →, ← and ⇒ represent the positions of the PCR primers FP1, RP1 and GFPF122, respectively. Large arrows (⇨) and boxes (□) denote the *p34.8* ORF and *p34.8* promoter, respectively. The dotted large arrow (⇨) and box (⊡) represent the GFP gene ORF and polyhedrin promoter, respectively. Vertical zigzag lines denote a broken *p34.8* ORF. (D) P34·8 is the amino acid sequence of P34·8. P34·8⁻ is the amino acid sequence of the P34·8 deletion mutant. The dashed line represents deleted amino acids. The locations of the conserved domains are shown.

site was blunted with Klenow) sites of pBlueBac4.5 (Invitrogen) to generate pBlueGFP (Fig. 1B). The fragment *Pst*I-H (5·4 kb), which contained *p34.8* of AcUW1-LacZ, was cloned into pUC18 (pAcPstH, Fig. 1A). This plasmid was cleaved with *Not*I (218 bp downstream of the *p34.8* ORF translation initiation codon ATG) and blunted with Klenow. The plasmid pBlueGFP was digested with *Eco*RV and *Hin*dIII, and blunted with Klenow to retrieve the polyhedrin promoter for GFP expression. The GFP fragment was gel purified and cloned into the blunted *Not*I site of pAcPstH. The transfer vector (pAcGFP), carrying 2·3 kb and 3·1 kb flanking DNA, was sequenced (Fig. 1B).

SF-21 cells were co-transfected with pAcGFP and AcUW1-LacZ DNAs (O'Reilly *et al.*, 1992). Plaque purification was carried out to isolate putative recombinants with GFP inserts. Expression of GFP as well as PCR amplification, restriction endonuclease (REN) analysis, DNA sequencing, and Southern and Northern blot analysis confirmed the authenticity of the recombinant viruses. The PCR primers used to amplify DNA from GFP plaques were located 20 bp downstream of the *p34.8* ORF ATG translation initiation codon and 100 bp downstream of the TAA termination signal (forward primer, FP1, 5′ CAT TGT TTG CTG CGA TTC AC 3′; reverse primer, RP1, 5′ GCA TAG CAG ACT ACG AAT TTG 3′). AcGFP was propagated in SF-21 cells and analysed by REN and Southern blot hybridization.

To construct a transfer vector lacking all the conserved *p34.8* domains, pPstH was digested with *Xba*I and blunted with Klenow. It was then cut with *Sal*I to retrieve a 1·7 kb fragment encoding the C terminus of P34.8. The plasmid pAcGFP was digested with *Not*I, and blunted. It was then cut with *Sal*I to retrieve a 5·8 kb fragment encoding the N terminus of P34.8 and part of a pUC18 sequence. The two fragments (1·7 and 5·8 kb) were ligated, propagated in DH5α cells and the clone generated termed pAcp34.8⁻ (Fig. 1C). SF-21 cells were co-transfected with pAcp34.8⁻ and AcGFP DNAs. White plaques containing putative recombinants lacking all the five conserved domains were isolated and initially screened by PCR as indicated above.

■ **REN, Southern and transcription analysis.** AcUW1-LacZ, AcGFP and pAcp34.8⁻ DNAs were digested with *Pst*I, separated on 0·7% agarose gels and transferred onto nylon membranes (Chomczynski, 1992). The 0·5 kb *Not*I–*Xba*I fragment internal to the *p34.8* ORF was labelled with ^{32}P by the random primer extension method (Sambrook *et al.*, 1989) and used as a probe in Southern blot analysis. For Northern blot analysis, SF-21 cells were infected, total RNA was extracted at various times thereafter (Chomczynski & Sacchi, 1987) and analysed (5 μg) on 1% denaturing formaldehyde–agarose gels. It was transferred to a nylon membrane and probed with ^{32}P-labelled *p34.8* ORF or with the *Not*I–*Xba*I DNA fragment. In RT–PCR analysis for transcription, a total

RNA sample obtained at 36 h post-infection was treated with RNase-free DNase (Promega). The same pair of primers (FP1 and RP1) employed in the PCR screening of viral recombinants was used in the RT–PCR to detect transcripts from the *p34.8* promoter. The reverse primer (RP1) and a 23-mer forward primer (GFPF122) starting at 122 bp from ATG of the GFP ORF were used in the RT–PCR to analyse transcripts initiated by the polyhedrin promoter. Negative controls not containing AMV reverse transcriptase were included. An Access RT–PCR System from Promega was used in all the RT–PCR analyses. The products were analysed on a 0·7% agarose gel.

■ **Bioassay and viral kinetics.** OBs of AcUW1-LacZ and pAcp34.8$^-$ were purified by differential and sucrose gradient centrifugation and suspended in distilled water at a concentration of 3×10^6 OBs/ml (Cheng *et al.*, 1990). Samples containing $1{\cdot}5 \times 10^4$, 3×10^4, 3×10^5, $1{\cdot}6 \times 10^6$ and 3×10^6 OBs/ml were used to determine the LD_{50} in larvae (Wang & McCathy, 1993). Briefly, 1 µl of virus suspension was applied onto a small diet plug and third-instar *Trichoplusia ni* larvae (50 animals per sample) were allowed to feed until all the diet was consumed, before transfer onto fresh diet. Larvae that did not consume all the diet within 24 h were discarded. Control larvae were treated with distilled water. Mortality was scored every day after infection until control larvae pupated. The dose-mortality data were analysed with the aid of the POLO-PC computer program (Le Ora Software Inc., Berkeley, CA, USA) which is based on the probit analysis method described by Finney (1971).

Kinetics of pAcp34.8$^-$ replication *in vitro* was compared with that of AcUW1-LacZ. SF-21 cells were infected with AcUW1-LacZ and pAcp34.8$^-$ at a multiplicity of 1 p.f.u. per cell. The virus was allowed to adsorb for 1 h and the cells were then washed with medium, re-furnished with fresh medium and incubated at 27 °C. Samples were removed from the infected cells at 12, 24, 36 and 48 h post-infection and assayed for budded viruses by the end-point dilution method (Summers & Smith, 1987). Differences in virus replication *in vitro* were analysed by regression analysis by using the Minitab computer program.

Results

Inactivation of *p34.8*

A transfer vector (pAcGFP), carrying the green fluorescence protein (GFP, 0·92 kb) gene under the control of the polyhedrin promoter, was constructed and used to insert the gene in the *Not*I site of *p34.8* (Methods, Fig. 1A). SF-21 cells were co-transfected with the transfer vector and AcUW1-LacZ DNAs and the putative recombinant progeny virus was initially observed by GFP expression. Progeny virus was plaque purified and green fluorescent plaques were further screened by PCR amplification to determine the proper insertion and orientation of the GFP gene (Fig. 2). One out of ten plaques yielded a single 1·9 kb PCR product suggestive of an authentic double-crossover recombinant free of the parental genotype. The rest of the plaques yielded both 1·0 kb and 1·9 kb PCR products indicative of a progeny contaminated with the parental genotype, or represented single-crossovers, or both (Fig. 2A). Recovery of a recombinant virus with disrupted *p34.8* suggested that this gene is not essential for virus replication. Sequencing of pAcGFP revealed that the insertion resulted in the introduction of three stop codons upstream of the GFP gene. However, another ORF that contained the five conserved domains was formed from an internal *p34.8* ATG codon just downstream of the TAA of the GFP gene (Fig. 1B). We could not rule out the possibility that the new ORF could somehow still be expressed. To exclude this possibility, it was decided to delete most of the *p34.8* ORF, including the five conserved domains. A second transfer vector based on this locus, minus the internal 531 bp *Not*I–*Xba*I fragment, was constructed (pAcp34.8$^-$, Fig. 1C, Methods). The deletion encompassed all the five conserved domains. SF-21 cells were co-transfected with this vector and AcGFP viral DNAs. The anticipated deletion mutant would have lost the gene encoding GFP as well as 531 bp from the *p34.8* ORF. Progeny virus was plaque purified as GFP$^-$ phenotype (Fig. 1C). A background of phenotypes expressing GFP was also observed. PCR amplification using a pair of *p34.8* primers (FP1 and RP1) resulted in the expected 470 bp long product (Fig. 1C, Fig. 2B). Amplification of the same region in AcUW1-LacZ with intact

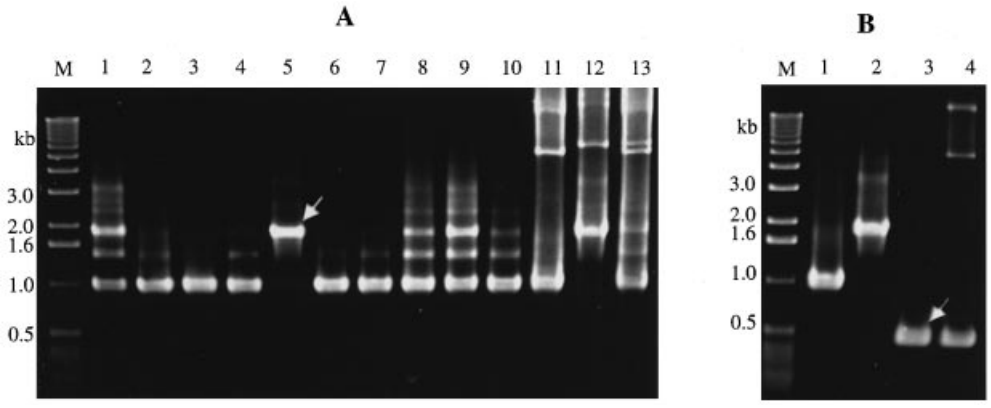

Fig. 2. PCR screening of putative recombinant AcUW1-LacZ. (A) Insertion of the gene encoding GFP. (B) Deletion of the gene encoding GFP and 531 bp from the *p34.8* locus. (A) Lanes 1–10 are PCR products from different GFP$^+$ plaques. Lanes 11, 12 and 13 are PCR controls on transfer vectors pAcPstH, pAcGFP and a mixture of both, respectively. (B) Lanes 1, 2, 3 and 4 are PCR products from AcUW1-LacZ , AcGFP, a GFP$^-$ plaque and pAcp34.8$^-$, respectively. M represents 1 kb DNA ladder marker (BioLabs). Arrows point to PCR products from pure plaques. The high molecular mass bands in both A (lanes 11, 12 and 13) and B (lane 4) are the plasmid templates.

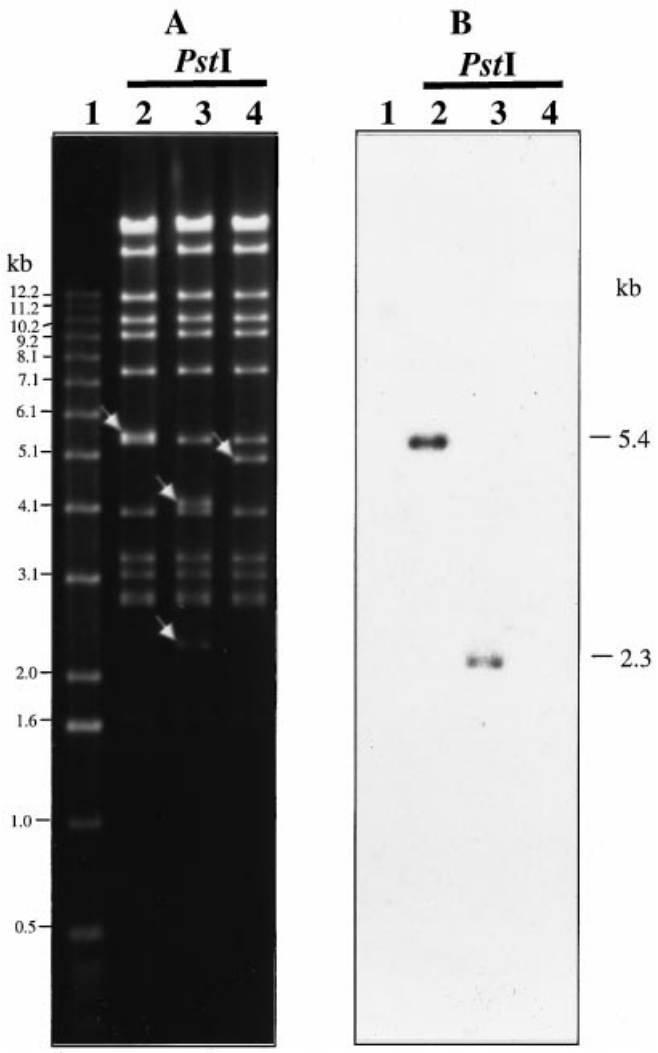

Fig. 3. REN (A) and Southern blot (B) analysis of AcUW1-LacZ , AcGFP and Acp34.8^{-} viral DNAs. (A) Digestion with *Pst*I and analysis on a 0·7% agarose gel. Arrows point to fragments that had changed mobility generated by rearrangement. (B) A Southern blot of DNA from panel (A) probed with ^{32}P random primer-labelled *Not*I–*Xba*I fragment containing the conserved domains sequence of *p34.8* from pAcPstH.

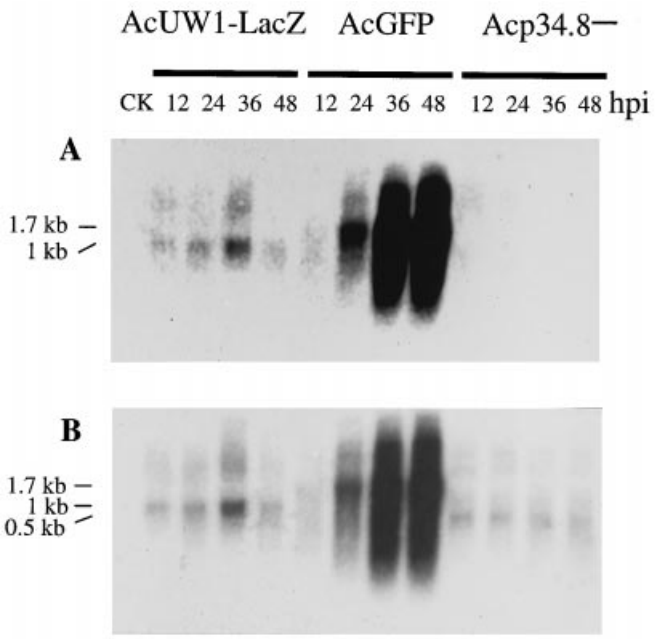

Fig. 4. Northern blot analysis of transcripts from AcUW1-LacZ, AcGFP and Acp34.8^{-} virus-infected SF-21 cells. The numbers indicate time (h) post-inoculation. (A) Blot probed with ^{32}P random primer-labelled *Not*I–*Xba*I from pAcPstH. (B) Blot probed with ^{32}P random primer-labelled PCR product of the *p34.8* ORF.

p34.8 yielded exclusively a 1 kb product, compared to 1·9 kb expected for *p34.8* interrupted with GFP (Fig. 2B). This showed that during homologous recombination between the transfer vector and AcGFP virus, a double-crossover had occurred which deleted the GFP gene and all the five *p34.8* conserved domains. The 470 bp PCR product was cloned into pGEM-T vector (Promega) and sequenced (Fig. 1C). The sequence confirmed that all the conserved domains were absent from the Acp34.8^{-} viral genome.

Restriction endonuclease analysis of AcGFP and Acp34.8^{-} substantiated the PCR screening data that a double-crossover event resulted in the formation of the latter virus. When the gene encoding GFP was inserted into the *p34.8* locus in the AcUW1-LacZ 5·4 kb *Pst*I-H fragment, two new *Pst*I fragments of 4·1 and 2·3 kb were generated (Fig. 3A). The 4·1 kb fragment contained the 0·92 kb GFP gene insert. In the deletion mutant, Acp34.8^{-}, the *Pst*I-H fragment had lost 0·531 kb from *p34.8* giving rise to a new 4·9 kb *Pst*I fragment (Fig. 3A). The additional *Pst*I site located after the GFP stop codon in the AcGFP genome was from the transfer vector pAcGFP derived from the multiple cloning site of pBlueBac4.5 (Fig. 1B). The *Pst*I restriction data were further confirmed by digestion of the viral genomes with *Eco*RI (data not shown).

Viral DNAs digested with *Pst*I (Fig. 3A) were blotted onto a nylon membrane and probed with ^{32}P-labelled 0·53 kb *Not*I–*Xba*I fragment from the *p34.8* ORF. The probe hybridized to the AcUW1-LacZ fragment *Pst*I-H and to the AcGFP 2·3 kb fragment but not to any of the Acp34.8^{-} DNA fragments (Fig. 3B). These data clearly confirm that the conserved domains of *p34.8* were deleted from the latter virus. Hybridization to *Eco*RI-digested viral genomes confirmed the above results (data not shown).

Northern blot analysis

Hybridization of ^{32}P-labelled *Not*I–*Xba*I fragment to Northern blots detected transcripts in SF-21cells infected with AcUW1-LacZ and AcGFP but not in cells infected with Acp34.8^{-} (Fig. 4A). The size of the transcripts from AcUW1-LacZ and AcGFP were 1 and 1·7 kb, respectively. The larger size of the transcripts in cells infected with AcGFP supports the

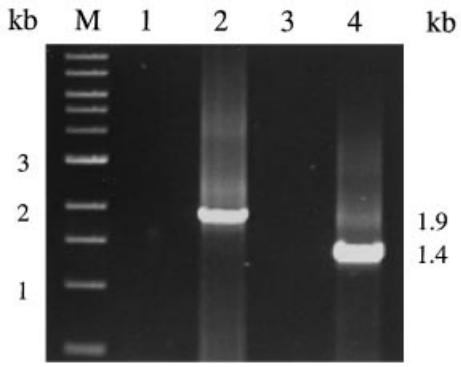

Fig. 5. RT–PCR analysis. Total RNA isolated at 36 h post-infection was used as template by AMV reverse transcriptase. Lanes 1 and 2 are RT–PCR products from a pair of primers in the *p34.8* sequence (FP1 and RP1). Lanes 3 and 4 are products from a forward primer in the GFP ORF sequence (GFPF122) and a reverse primer in the *p34.8* sequence (RP1). Lanes 1 and 3 are controls not containing AMV reverse transcriptase. M, 1 kb DNA marker (BioLabs).

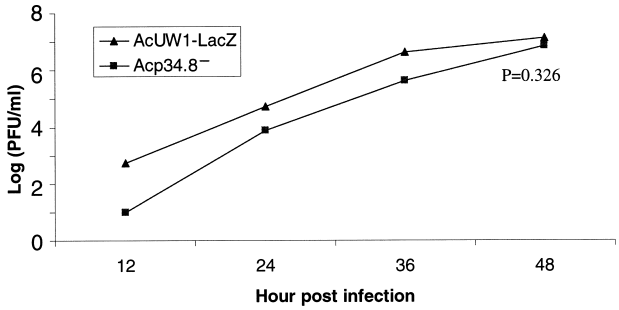

Fig. 6. Growth of AcUW1-LacZ and Acp34.8⁻ in SF-21 cells. SF-21 cells were infected with AcUW1-LacZ and Acp34.8⁻ at a multiplicity of 1 p.f.u. per cell. Samples were taken from the infected cells at 12, 24, 36 and 48 h post-infection and assayed for budded viruses by the end-point dilution method. Differences in virus replication were analysed by regression analysis.

evidence that the gene encoding GFP was inserted into the *p34.8* ORF. The abundant transcription from this locus in AcGFP is attributed to the polyhedrin promoter, which is clearly much stronger than that of *p34.8*. Transcription from *p34.8* promoter was obscured by the abundance of transcripts generated by the polyhedrin promoter. RT–PCR analysis corroborated the data from Northern hybridization (Fig. 5). Using a pair of primers (FP1 and RP1) from *p34.8* sequence, a 1·9 kb RT–PCR product was amplified. When the GFP forward primer (GFPF122) and *p34.8* reverse primer (RP1) were used, an expected 1·4 kb RT–PCR product was produced (Fig. 5). Controls did not show any products indicating that the RT–PCR products were from mRNA and not from viral DNA (Fig. 5). When the total ^{32}P-labelled *p34.8* ORF was used as a probe in the Northern blots, the mRNAs from AcUW1-LacZ, AcGFP and Acp34.8⁻ viruses were 1·0, 1·7 and 0·5 kb, respectively (Fig. 4 B). The lack of transcripts from Acp34.8⁻ and the size of the transcripts from the three viruses confirmed that the *Not*I–*Xba*I part of the ORF had been deleted successfully (Fig. 4 A).

LD_{50} bioassays and viral kinetics

Since entomopoxvirus fusolin has been reported to have an enhancing effect on baculoviruses, we were interested to find out if its homologue P34.8 had a similar effect. Inactivation of *p34.8* offers the possibility of investigating the effect on infectivity of OBs to larvae or on the kinetics of virus replication in tissue culture. The LD_{50} of purified Acp34.8⁻ OBs was determined in third-instar *T. ni* larvae by using the diet plug method (Wang & McCathy, 1993). Parental AcUW1-LacZ was used as a control. No significant difference in LD_{50} was observed between the deletion mutant and the parental viruses (Table 1). It has been reported previously that AcMNPV is the only baculovirus identified so far where P34.8 was associated with the OBs (Gross *et al.*, 1993; Phanis *et al.*, 1999; Vialard *et al.*, 1990). The lack of difference in the infectivity of OBs from the two viruses could also be due to the fact that only very small amounts of the protein are associated with the OBs (Gross *et al.*, 1993; Vialard *et al.*, 1990). In the kinetics of replication analysis, no significant difference in the slopes of the growth curves between AcUW1-LacZ and Acp34.8⁻ was detected ($P = 0{\cdot}326$). This indicated that inactivation of *p34.8* had no effect on virus replication in SF-21 cells (Fig. 6).

Table 1. Dose-mortality response of third-instar *T. ni* to AcUW1-LacZ and Acp34.8⁻

LD_{10}, LD_{50}, LD_{90} and associated statistics were calculated with the computer program POLO-PC (Le Ora Software). Numbers in parentheses represent lower and upper 95% confidence limits.

Virus	Dose (OBs per larva)			Slope
	LD_{10}	LD_{50}*	LD_{90}	
AcUW1-LacZ	30·7 (13·0–58·3)	283·9 (163·3–488·0)	2626·3 (1408·9–5971·8)	1·266
Acp34.8⁻	34·4 (14·5–65·6)	318·4 (182·8–548·5)	2945·2 (1582·1–6673·3)	1·392

* No significant difference was found in LD_{50} values between AcUW1-LacZ and Acp34.8⁻.

Discussion

This study was initially undertaken to determine if the EPV gene encoding fusolin could substitute for *p34.8* or other homologues in baculoviruses. Unlike the fusolin gene, it was assumed that *p34.8* was probably essential for baculovirus replication because *p34.8*-null mutants could not be isolated (Wu & Miller, 1989). Since the codon usage of this gene favours A and T residues (Wu & Miller, 1989), similar to most entomopoxvirus genes, it is likely that during evolution the gene was transferred from EPVs to baculoviruses. If this is true, then could the fusolin gene substitute for *p34.8* in baculoviruses? Recently, this gene and its encoded product from CfDEFNPV was characterized and it was shown that the gene product accumulates as bipyramidal crystals in the cytoplasm of infected cells (Gross *et al.*, 1993; Li *et al.*, 2000). The question arose as to the essential function of a cytoplasmic protein in baculovirus replication. For this reason, we decided to ascertain the requirement for *p34.8* in baculoviruses before attempting to swap it with an EPV fusolin gene.

We attempted to inactivate *p34.8* in AcUW1-LacZ with an insertion of the gene encoding GFP (0·92 kb). PCR amplification of the *p34.8* locus revealed that a recombinant virus with a GFP insert could be generated. We also deleted all the five conserved domains from the ORF (531 bp). PCR amplification unequivocally identified a virus with a double-crossover substitution that was free from contaminating parental or single-crossover genotypes (Fig. 2B). No viable virus with inactivated *p34.8* was isolated by previous investigators probably because their sample size (eight plaques) was too small and they did not use PCR to identify mutant viruses (Wu & Miller, 1989). In the experiments reported here, PCR amplification was invaluable in identifying authentic mutants and their purity. Transcripts from the *p34.8* deletion mutants had the expected size of 0·5 kb. The presence of viable virus with inactivated *p34.8* was authenticated by PCR amplification, REN and Southern, Northern and RT–PCR analyses. Also, sequencing of the *Pst*I fragment generated by the deletion demonstrated that 531 bp were missing from the ORF (Fig. 1C, D).

Inactivation of *p34.8* did not appear to have an effect on the viral LD_{50} in *T. ni* larvae or on growth kinetics in SF-21 cells (Table 1, Fig. 6). It could be that too little protein is associated with the OBs to have a demonstrable effect on LD_{50}. The plentiful expression of the protein by CfDEFNPV (Li *et al.*, 2000) should help in determining the function of this protein in baculoviruses.

In conclusion, two independent criteria were used to show that *p34.8* was not essential for virus replication. The gene was inactivated with an insert encoding GFP and by deleting the large portion of the ORF containing all the conserved domains. In both cases, viable mutants were isolated and authenticated. This locus in the viral genome could, therefore, potentially provide another site for the engineering of baculoviruses in cases where other non-essential loci, such as those of polyhedrin or *p10*, needed to remain in the virus.

We would like to thank Hilary Lauzon and Tim Ladd for assistance in DNA sequencing and Dr Richard Fleming for help in statistical analysis. This research was supported by Canadian NSERC strategic grant number STPO193108.

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Received 14 August 2000; Accepted 20 October 2000