

Expression of cauliflower mosaic virus gene I in insect cells using a novel polyhedrin-based baculovirus expression vector

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An improved polyhedrin-based baculovirus expression vector was constructed to expedite distinguishing infections by putative baculovirus recombinants from infections by wild-type (wt) baculovirus. The vector utilizes the *Escherichia coli* β -galactosidase gene (*lacZ*) as a genetic marker for positive recombination between wt *Autographa californica* nuclear polyhedrosis virus and the baculovirus transfer vector. The marker gene/expression cassette was constructed so that *lacZ* and the deleted polyhedrin gene were transcribed in opposite orientations, both terminating in a simian virus 40 DNA fragment which acts as a bidirectional terminator. In the constructed vector, *lacZ* is transcribed from the *Drosophila melanogaster* heat-shock promoter (*hsp70*), which is constitutively expressed in baculovirus-infected *Spodoptera frugiperda* (Sf) cells, thereby making the site of the deleted polyhedrin gene

available for the insertion and expression of foreign genes under the control of the polyhedrin promoter. Recombinant baculoviruses are readily selected in plaque assays by the development of a blue colour upon the addition of X-Gal. The colour selection renders the retrieval of recombinants less dependent on a high frequency of recombination between the transfer vector and wt baculovirus DNA. The usefulness of this new vector was illustrated by expressing gene I of cauliflower mosaic virus, which encodes a protein of M_r 46 000. Expression of gene I was at the same level as in cells infected with a conventional polyhedrin-based expression vector. Gene I protein formed large hollow fibre-like structures in the cytoplasm of infected Sf cells. This is the first plant virus protein to be expressed in insect cells by a recombinant baculovirus.

Introduction

Baculoviruses have attracted wide attention, not only for their ability to control insect pests (Granados & Federici, 1986) but also for their use as eukaryotic vectors for the expression of foreign genes (Luckow & Summers, 1988; Miller, 1988, 1989; Maeda, 1989). When expressed in this system, recombinant proteins of various origins appeared to be chemically, antigenically, immunologically and functionally similar, if not identical, to the authentic proteins. However, plant virus proteins have not yet been expressed in this system.

Autographa californica nuclear polyhedrosis virus (AcNPV) is the type member of the family Baculoviridae (Matthews, 1982). The viral genome consists of circular dsDNA of 128 kb (Cochran *et al.*, 1982). Based upon their temporal expression during infection, four classes of genes have been identified (Kelly, 1982); the class of very late genes is composed of the highly expressed

polyhedrin and p10 genes, the products of which are dispensable for virus replication. Polyhedrin is the major component of the large protein crystals, or polyhedra, produced at the end of the infection, whereas the function of the p10 protein is unknown, but it may be involved in cell lysis (Williams *et al.*, 1989).

The baculovirus expression system is based on the allelic replacement of the polyhedrin gene by heterologous genes. Up to now, only polyhedrin-based expression vectors have been in use. These are constructed via transfer vectors, which, in addition to pUC sequences and a prokaryotic replication origin, contain all regulatory elements of the polyhedrin gene, i.e. the promoter and the poly(A) addition signal, as well as extended 5' and 3' flanking sequences. In these transfer vectors, the polyhedrin gene has been replaced by, or fused to, the foreign gene of interest. Recombinant viruses are obtained following homologous recombination between the 5' and 3' extended flanking sequences

of the polyhedrin gene of the transfer vector and wild-type (wt) viral DNA.

The percentage of recombinant viruses obtained by this recombination event is not very high (up to 0.5%). Several methods have been described to isolate recombinant viruses: (i) light microscopy to screen for polyhedra-negative plaques (Smith *et al.*, 1983); (ii) dot blot or plaque hybridization with foreign nucleic acid probes (Summers & Smith, 1987; Pen *et al.*, 1989); (iii) *in situ* detection using antibodies (Capone, 1989); (iv) use of a polyhedra-negative mutant virus and a transfer vector, which contains a duplicated polyhedrin promoter and a polyhedrin gene, to generate polyhedra-positive recombinant viruses (Emery & Bishop, 1987). To simplify the selection procedure for recombinants we have developed a new polyhedrin-based transfer vector. The recognition of putative recombinants relies upon the constitutive expression of the bacterial *lacZ* gene and is completely independent of viral gene expression.

Cauliflower mosaic virus (caulimovirus group; CaMV) has a dsDNA genome which contains eight open reading frames (ORFs). Expression of ORFs I to VI has been detected *in vivo* and functional roles have been assigned to each of the encoded proteins (Covey, 1985; Maule, 1985); it is unknown whether ORF VII and VIII are expressed. The product of ORF I (P1) is thought to be involved in cell-to-cell spread of the virus (Linstead *et al.*, 1988) and is present in trace amounts in cell walls of infected plants (Albrecht *et al.*, 1988). Recently, it was shown that large amounts of P1 are present in tissues where movement of virus from infected to uninfected cells was most likely to have occurred (Maule *et al.*, 1989). To study the structure of P1 and its putative role in cell-to-cell spread in more detail, large amounts of protein are required; insect cells and baculovirus vectors were used as a means of doing this.

This paper describes the construction of a novel polyhedrin-based transfer vector carrying *lacZ* as a reporter gene and the expression of CaMV gene I by AcNPV recombinants. An M_r 46000 (46K) protein was identified as the P1 product of the CaMV gene I and it formed hollow fibre-like structures in infected insect cells.

Methods

Cells and virus. Maintenance of *Spodoptera frugiperda* (Sf; IPLB-SF21) insect cells (Vaughn *et al.*, 1977) and production of wt AcNPV strain E2 (Smith & Summers, 1978) have been described previously (Vlak & Odink, 1979). Virus was grown on cell monolayers, cultured at 28 °C in plastic tissue culture flasks in TNM-FH medium (Hink, 1970) supplemented with 10% foetal calf serum. Isolation and properties of recombinant AcNPV/*lacZ* are described by Summers & Smith (1987).

Competent cells of *Escherichia coli* strain DH5 α (Gibco-BRL) were

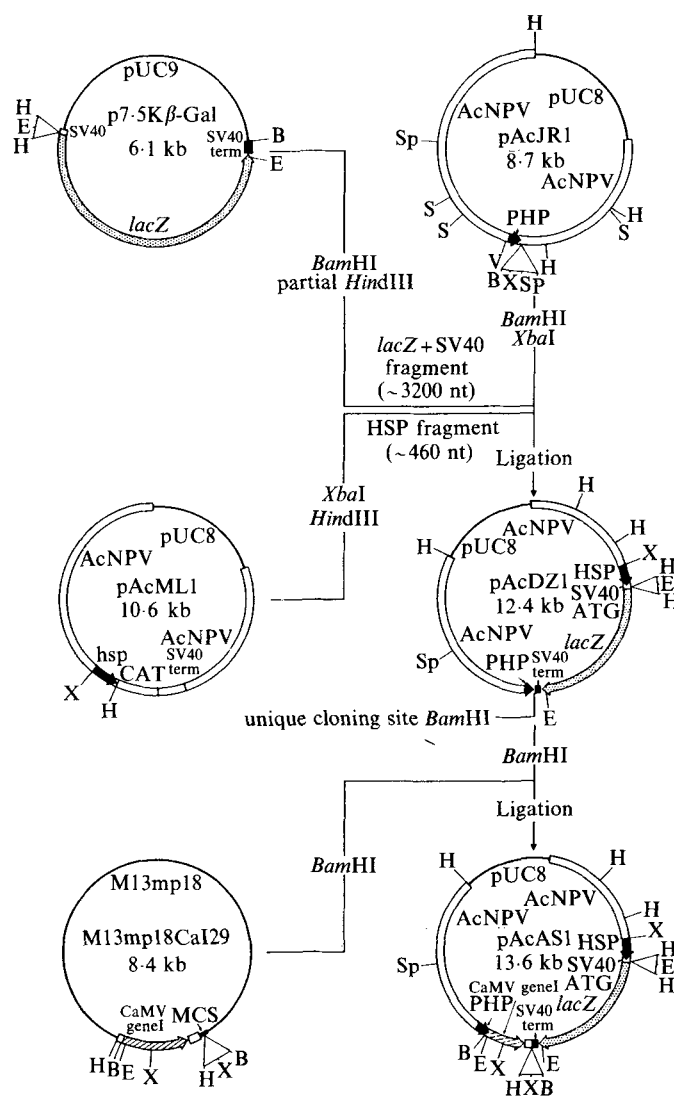


Fig. 1. Construction scheme of transfer vectors pAcDZ1 and pAcAS1. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sal*I; Sp, *Sph*I; X, *Xba*I sites. MCS, multiple cloning site. PHP, polyhedrin promoter; HSP, hsp70 promoter; CAT, chloramphenicol acetyltransferase gene; SV40 term, simian virus 40 terminator sequence.

used in plasmid DNA transformations according to the manufacturer's protocol.

DNA manipulations. All plasmid DNA recombination techniques were essentially as described by Maniatis *et al.* (1982) and Sambrook *et al.* (1989). Restriction enzymes, T4 DNA ligase and other DNA-modifying enzymes were purchased from Gibco-BRL.

Construction of transfer vectors pAcDZ1 pAcAS1 and pAcAM1

(i) *pAcDZ1 and pAcAS1.* The construction of transfer vectors pAcDZ1 and pAcAS1 is outlined in Fig. 1. The intermediate transfer vector pAcJR1 is a derivative of pAc610 (Luckow & Summers, 1988). The 1.9 kb *Sph*I–*Bam*HI fragment of pAc610, which contains the polyhedrin promoter, was replaced by the *Sph*I–*Bam*HI fragment of the same size from pAcRP23 (Possee & Howard, 1987) in order to obtain the complete polyhedrin promoter sequence.

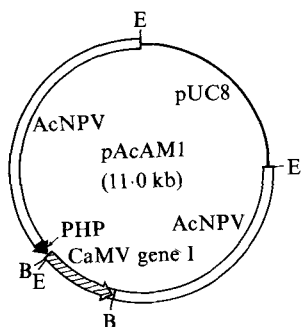


Fig. 2. Structure of transfer vector pAcAM1. B, *Bam*HI; E, *Eco*RI sites. PHP, polyhedrin promoter.

Plasmid pAcDZ1 was assembled from pAcJR1, p7-5K β -gal and pAcML1. Briefly, the construction was as follows. Plasmid p7-5K β -gal was digested to completion with *Bam*HI and partially with *Hind*III. A 3.2 kb *Bam*HI–*Hind*III fragment containing *lacZ* and simian virus 40 (SV40) termination signals was isolated. From plasmid pAcML1, a 460 nucleotide (nt) *Xba*I–*Hind*III fragment, encompassing the *Drosophila melanogaster* heat-shock promoter (hsp70), was isolated. Plasmid pAcJR1 provided a 8.7 kb *Bam*HI–*Xba*I fragment containing the polyhedrin promoter and its flanking baculovirus sequences. Ligation of these three fragments, followed by transformation, yielded transfer vector pAcDZ1. Plasmid p7-5K β -gal consists of the bacterial *lacZ* gene (Casadaban *et al.*, 1981) and the SV40 transcription initiation and termination sequences (Kalderon *et al.*, 1984). The terminator sequence contains polyadenylation signals in both strands. Plasmid pAcML1 was kindly provided by M.-J. van Lierop. The *Drosophila* hsp70 of this plasmid, obtained from plasmid pHSPCAT (a kind gift from H. Pelham, Cambridge, U.K.), originated from clone 132E3 (Karch *et al.*, 1981).

Plasmid pAcAS1 was derived from pAcDZ1 and the replicative form of M13mp18Ca129. The latter contained a *Hind*III–*Sca*I (nt 321 to 1513) fragment of CaMV strain Cabb-S (Franck *et al.*, 1980) encompassing the entire gene I. Site-specific mutagenesis (Kunkel *et al.*, 1987) was used to introduce a *Bam*HI site 18 nt upstream of the gene I ATG translational start, such that gene I could be isolated as a 1.2 kb *Bam*HI fragment for ligation into the unique *Bam*HI site of pAcDZ1. The resulting vector, designated pAcAS1, contained CaMV gene I inserted in the correct orientation behind the polyhedrin promoter.

(ii) *pAcAM1* (Fig. 2). A 1.2 kb *Bam*HI fragment, containing gene I of CaMV, was isolated from the replicative form of M13mp18Ca129 (see Fig. 1). This *Bam*HI fragment was ligated into the *Bam*HI site of transfer vector pAcRP23 (Possee & Howard, 1987), resulting in transfer vector pAcAM1. The correct orientation was verified by *Eco*RI digestion of transfer vector pAcAM1, using the asymmetric location of the *Eco*RI site in CaMV gene I as a marker.

Infection and transfection of insect cells. Sf cells seeded in 35 mm Petri dishes were cotransfected with 1 μ g wt AcNPV DNA and 10 μ g plasmid DNA (purified by sedimentation to equilibrium in CsCl) using the calcium phosphate technique, essentially as described by Summers & Smith (1987) with some minor modifications (Vlak *et al.*, 1988). After 5 days of plaque development, recombinant AcAM1 was obtained by screening for polyhedra-negative plaques. Recombinants AcDZ1 and AcAS1 were detected by adding 25 μ g 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) to the Petri dishes. Recombinant viruses which induced the development of a blue colour upon addition of X-Gal were selected. Each putative recombinant was subjected to at least three rounds of plaque purification to obtain genetic homogeneity.

Sf cells were infected with the non-occluded form of the virus at a multiplicity of 10 TCID₅₀ units per cell.

DNA and protein analysis. Viral DNA, obtained from non-occluded virus and plasmid DNA, was isolated essentially as described by Vlak *et al.* (1988) and Maniatis *et al.* (1982), respectively. DNA digestions were performed with restriction enzymes (Gibco-BRL) and analysed on 0.7% agarose gels.

Infected and mock-infected Sf cells (6×10^6) were washed three times in phosphate-buffered saline (PBS; 8% NaCl, 0.2% KCl, 1.15% Na₂HPO₄, 0.2% KH₂PO₄ pH 7.3), resuspended and boiled in 10 mM-Tris-HCl pH 8.0, 1 mM-EDTA, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol and 0.001% (w/v) bromophenol blue. Protein extracts of CaMV-infected turnip leaves were prepared as described by Harker *et al.* (1987). Protein samples were analysed by electrophoresis in 10% SDS-polyacrylamide gels (Laemmli, 1970). Gels were stained with silver (Maniatis *et al.*, 1982; Sambrook *et al.*, 1989) or used for immunoblotting.

Immunoblotting and analysis were performed as described by Harker *et al.* (1987). Polyclonal rabbit antisera raised against a bacterial fusion product of β -galactosidase and CaMV P1 (Harker *et al.*, 1987) or P1 purified by SDS-PAGE from AcAM1-infected insect cells, were used for detection of CaMV P1 on immunoblots. Protein from the 46K band was purified from gels by electroelution and injected into rabbits.

Electron microscopy. Sf cells were infected with wt or recombinant AcNPV, at a multiplicity of 20 TCID₅₀ units per cell, and incubated for 54 h. The cells were processed for electron microscopy and immunocytochemistry as described by van Lent *et al.* (1990).

Results

Construction of transfer vectors pAcDZ1, pAcAS1 and pAcAM1

Transfer vector pAcDZ1 was obtained after a one-step ligation of three different fragments, obtained from pAcML1, p7-5K β -gal and pAcJR1 (Fig. 1). This vector has the following characteristics: (i) *Drosophila* hsp70 drives the expression of the *lacZ* gene in a constitutive fashion; (ii) transcription of *lacZ* is in the opposite direction to the engineered transcript, utilizing the polyhedrin promoter; (iii) transcripts of both hsp70 and the polyhedrin promoter will be polyadenylated on opposite strands by the signals present in the SV40 termination sequences.

To test the performance of this newly designed transfer vector, gene I of CaMV was cloned as a 1.2 kb *Bam*HI fragment into the unique *Bam*HI cloning site of pAcDZ1, to give transfer vector pAcAS1. Both pAcDZ1 and pAcAS1 were used to obtain AcNPV recombinants. Recombinant viruses were isolated and analysed to study the expression of CaMV gene I and *lacZ*.

Transfer vector pAcAM1 was derived from pAcRP23 (Possee & Howard, 1987), which has the flanking sequences of the polyhedrin gene and a deletion of 170 nt

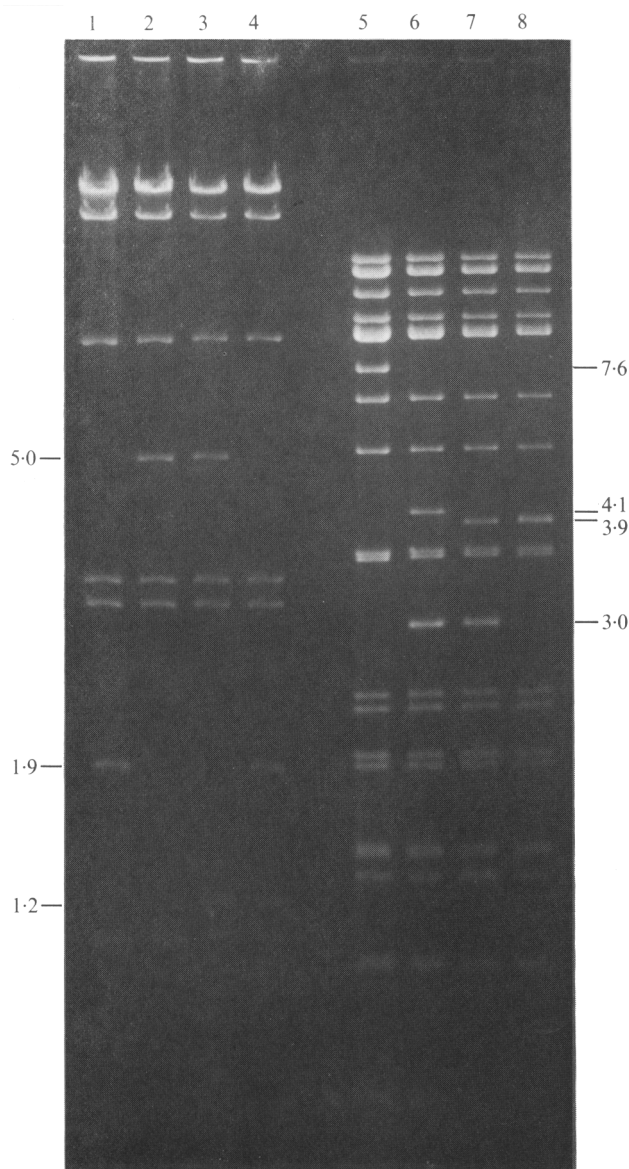


Fig. 3. Restriction endonuclease profiles of DNA fragments of wt AcNPV (wtAc) and three recombinants of AcNPV (AcDZ1, AcAS1 and AcAM1). Lanes 1 and 5, wtAc. Lanes 2 and 6, AcDZ1. Lanes 3 and 7, AcAS1. Lanes 4 and 8, AcAM1. Lanes 1 to 4 *Bam*HI digests, lanes 5 to 8 *Eco*RI digests. Differences in banding patterns are indicated by sizes (kb) to left and right of the figure.

from the N-terminal part of the polyhedrin gene, including the ATG. A 1.2 kb *Bam*HI fragment containing CaMV gene I, obtained from the replicative form of M13mp18CaI29, was inserted behind the polyhedrin promoter to give pAcAM1 (Fig. 2). Recombinant AcAM1 was selected by visual inspection for polyhedra-negative plaques.

DNA analysis of polyhedrin-minus recombinants AcDZ1, AcAS1 and AcAM1

Sf cells were transfected with either pAcDZ1, pAcAS1 or pAcAM1 and wt AcNPV DNA. Recombinants were selected by their polyhedra-negative appearance (AcAM1) or by blue coloration (AcDZ1 and AcAS1). After several rounds of plaque purification, viral DNA was isolated and analysed by restriction enzyme digestions using *Bam*HI and *Eco*RI (Fig. 3). The digestion patterns of the three different recombinants were compared with the equivalent patterns of wt AcNPV DNA. Fragment *Bam*HI-F (1.9 kb) of wt AcNPV is not present in recombinants AcDZ1 and AcAS1 (Fig. 3, lanes 1, 2 and 3) due to a deletion of 670 nt of the polyhedrin-coding region and insertion of foreign gene elements. Instead, a new fragment of 5.0 kb appeared in both recombinants consisting of a 3.7 kb reporter gene construct (*lacZ* and *hsp70*) and the remaining 1.3 kb of the *Bam*HI-F fragment. An additional fragment appeared in recombinants AcAS1 and AcAM1 (Fig. 3, lanes 3 and 4) which represents the total insert of CaMV gene I (1.2 kb). Recombinant AcAM1 displays a different DNA restriction pattern, due to the smaller deletion of the polyhedrin-coding region, which makes the *Bam*HI-F fragment only 170 nt smaller.

The *Eco*RI digestion patterns showed that, in comparison with wt AcNPV, the *Eco*RI-I fragment (7.6 kb) was absent from all three recombinants (Fig. 3, lanes 5, 6, 7 and 8). In the case of AcDZ1 three new fragments appeared. One fragment, of 4.1 kb, contained the polyhedrin promoter and upstream sequences of the *Eco*RI-T fragment, as well as the SV40 termination sequences and 40 nt encoding the carboxy terminus of *lacZ*. The two other fragments appear as a doublet (3 kb each) and consist of the *lacZ* fragment and the *hsp70* fragment and downstream sequences of the *Eco*RI-T fragment (minus the deletion of 670 nt of the polyhedrin gene). Recombinant AcAS1 shows a similar pattern, except that the 4.1 kb fragment of AcDZ1 was shortened to 3.9 kb and the *Eco*RI-T fragment became a doublet as a result of the insertion of CaMV gene I. Recombinant AcAM1 has a doublet at 3.9 kb, as a result of the introduction of CaMV gene I into the polyhedrin locus. The identity of *lacZ* and CaMV-specific inserts was confirmed by Southern blot hybridization (data not shown).

Protein analysis of polyhedrin recombinants AcDZ1, AcAS1 and AcAM1

Proteins were isolated from uninfected and recombinant or wt AcNPV-infected Sf cells 72 h post-infection and

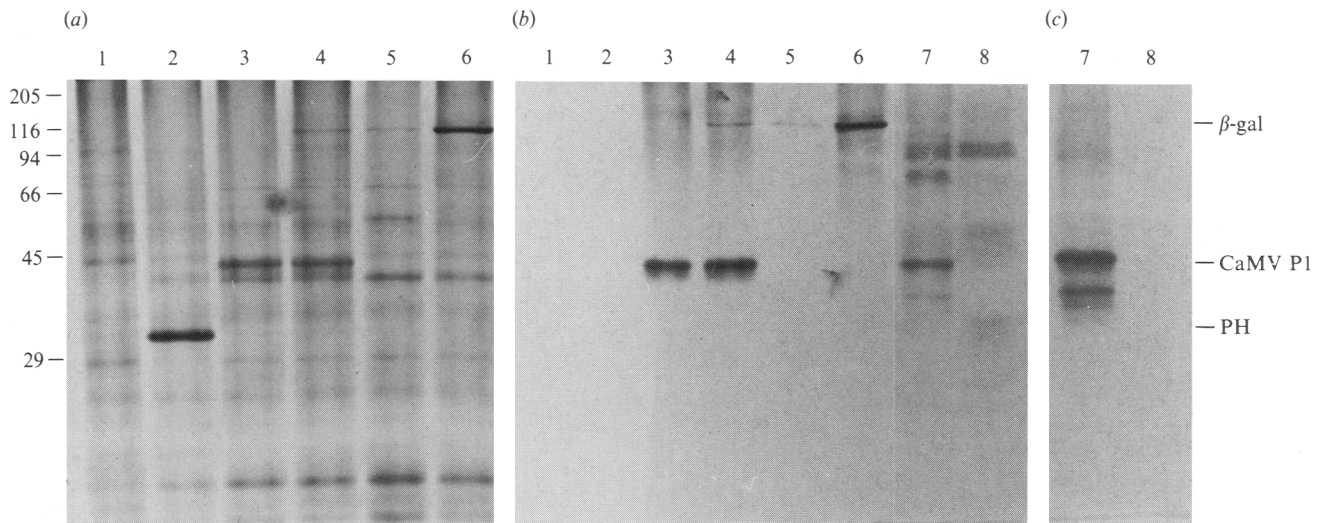


Fig. 4. Silver-stained SDS-polyacrylamide gel (a) and immunoblot analysis (b and c) of proteins extracted from uninfected Sf cells (lanes 1), wtAc- (lanes 2), AcAM1- (lanes 3), AcAS1- (lanes 4), AcDZ1- (lanes 5) or AcNPV/*lacZ*- (lanes 6) infected Sf cells, and CaMV-infected (lanes 7) or healthy (lanes 8) turnip leaves. Immunoblot analysis was performed with polyclonal rabbit antisera raised against a β -gal-P1 fusion protein (b) and with polyclonal rabbit antisera raised against P1, isolated from AcAS1-infected cells (c). The positions of β -galactosidase (116K), CaMV P1 (46K) and polyhedrin (30K) are shown to the right. Numbers to the left are M_r s ($\times 10^{-3}$) of marker proteins.

analysed in SDS-polyacrylamide gels. Silver staining of the gels showed that the polyhedrin recombinants AcAM1 and AcAS1 both produce a protein of 46K in equal amounts (Fig. 4a, lanes 3 and 4). In both cases the polyhedrin promoter drives the expression of CaMV gene I. Compared with wt AcNPV-infected cells, the level of expression of the 46K protein was in the same order of magnitude as polyhedrin (Fig. 4a, lanes 2, 3 and 4). Recombinants AcAS1, AcDZ1 and AcNPV/*lacZ* also express a protein of 116K (Fig. 4a, lanes 4, 5 and 6). The identity of this protein was confirmed in immunoblot analysis as β -galactosidase (Fig. 4b, lanes 4, 5 and 6). The amount of 116K protein was much greater when expressed under the control of the polyhedrin promoter (Fig. 4a, lane 6) than when under the control of *Drosophila* hsp70. In infected insect cells, hsp70 was turned on immediately after infection and constitutively expressed *lacZ*, which contrasts with the polyhedrin promoter which is turned on much later in infection (data not shown).

Immunoblot analysis showed that the rabbit polyclonal antisera raised against the product of gene I of CaMV reacted with a 46K protein produced by AcAM1- and AcAS1-infected insect cells (Fig. 4b, lanes 3 and 4) and with a product of the same size from infected turnip tissue (Fig. 4, lanes 7). This antiserum was raised against a fusion product of β -galactosidase and the gene I product so it also reacted with the 116K protein (Fig. 4b, lanes 4, 5 and 6). Antiserum raised against the 46K

protein originating from insect cells reacted with a protein of similar size in leaf extracts of CaMV-infected turnip (Fig. 4c, lane 7).

Cytopathology of polyhedrin recombinants and wt AcNPV-infected cells

Sf cells were infected with either wt AcNPV or recombinant AcAS1. After 54 h, cells were prepared for electron microscopy (Fig. 5). As expected, no polyhedra could be observed in the nuclei of cells infected with recombinant AcAS1, in contrast to those of wt AcNPV-infected cells (Fig. 5b and a, respectively). The formation and appearance of electron-dense 'spacers' and fibrillar structures was similar in cells infected with either type of virus. In the cytoplasm of cells infected with AcAS1, hollow fibre-like structures were present in large numbers (Fig. 5c). Occasionally, these structures were also present in the nuclei. The external diameter of the fibre-like structures was about 22 nm and the inner diameter about 10 nm. Immunocytochemistry of ultrathin sections of cells infected with AcAS1 with antisera directed against the CaMV gene I product using Protein A-gold (Fig. 5c) showed strong labelling over the hollow fibre-like structures, whereas the background reaction in the cytoplasm was low. When the sections were incubated with antiserum against p10, the fibrillar structures were labelled (van der Wilk *et al.*, 1987), whereas no label was found associated with the hollow fibre-like structures.

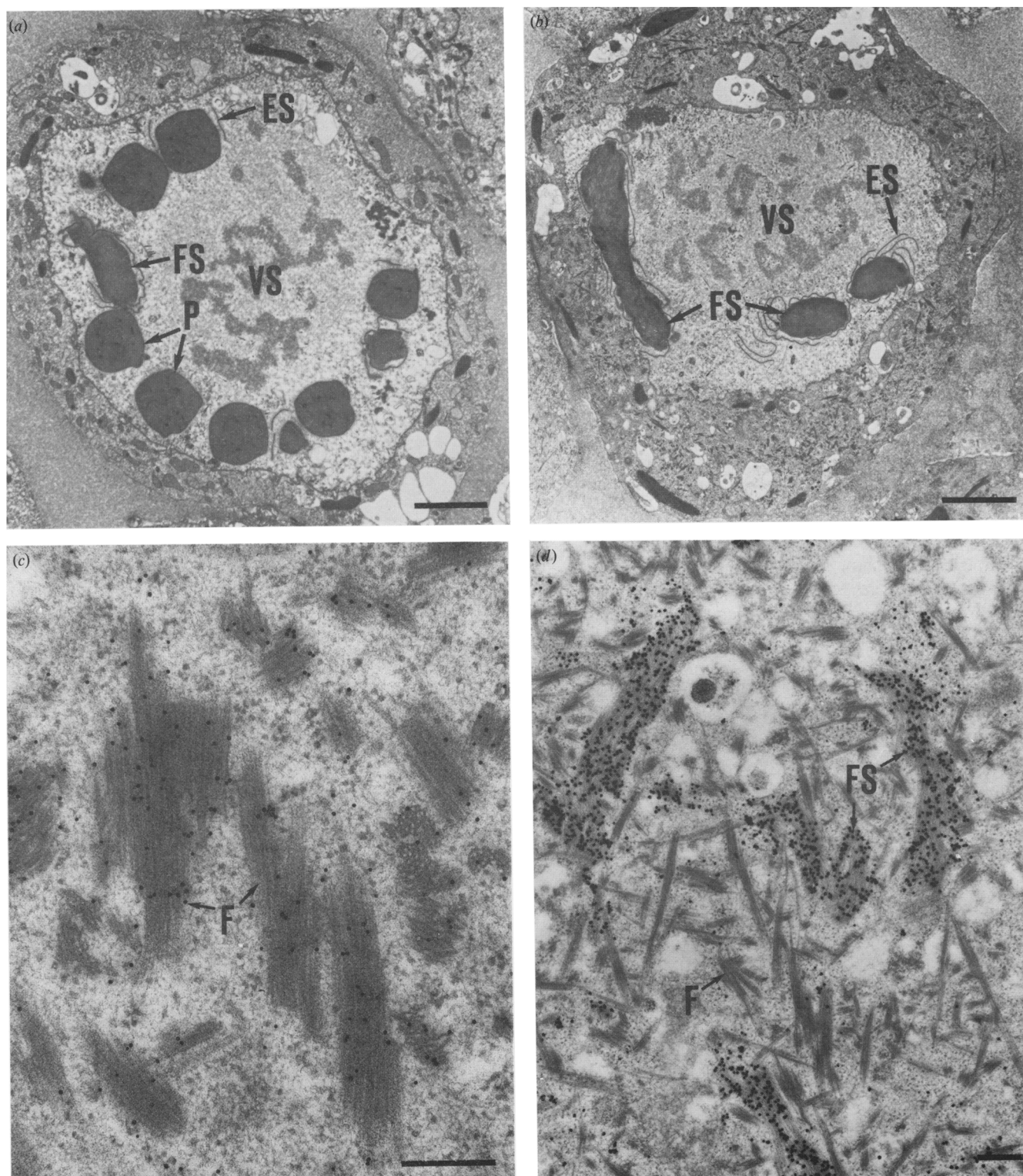


Fig. 5. Electron micrographs of Sf cells infected with (a) wt AcNPV and (b, c, d) AcAS1. Sections were treated with antiserum raised against (c) the β -gal-P1 fusion protein and (d) p10, and complexed with (c) Protein A-gold or (d) enhanced with silver. The antiserum against the β -gal-P1 fusion protein reacted predominantly with the hollow fibre-like structures (F) in the cytoplasm. Arrowheads point to cross-sections of the fibre-like structures. ES, electron-dense 'spacers'; FS, fibrillar structures; P, polyhedra; VS, virogenic stroma. Bar markers represent (a, b) 2 μ m and (c, d) 300 nm.

Discussion

The baculovirus expression vector system is based on the allelic replacement of the polyhedrin gene by foreign genes of interest. Various methods have been described to select for recombinant viruses, among which are dot blot and plaque hybridization using either nucleic acid or antisera as probes. Up to now, the screening procedures for recombinant viruses have been cumbersome and require considerable experimental skill. To facilitate screening of polyhedrin-negative plaques, a new polyhedrin-based transfer vector was developed. Previous experiments had shown that the *D. melanogaster* hsp70 was constitutively expressed in Sf-21 cells, as assayed by chloramphenicol acetyltransferase expression (M. J. van Lierop, personal communication). Based on this observation, transfer vector pAcDZ1 was constructed which constitutively expressed the bacterial *lacZ* gene under the control of the hsp70 promoter. This unit functioned as a marker gene to screen for recombinants, whereas the polyhedrin promoter was still available for foreign gene expression. Since the direction of transcription of the *lacZ* gene from the hsp70 promoter was in the opposite orientation in comparison with expression dictated by the polyhedrin promoter, an SV40 termination signal was introduced to terminate transcription and to prevent considerable overlap of two opposite transcripts.

Screening for recombinant viruses was by addition of the β -galactosidase indicator X-Gal to the overlay of the plaque assays and the appearance of blue plaques. This new expression vector system can be used to detect recombinants when the recombination frequency is low.

To test the suitability of this novel transfer vector, gene I of CaMV was inserted behind the polyhedrin promoter. As is evident from the comparison of expression of recombinant AcAM1 and AcAS1 (Fig. 4, lanes 3 and 4, respectively), CaMV gene I expression is not affected by the expression of *lacZ*. Similar amounts of 46K protein are produced by both recombinants. Compared with wt AcNPV (Fig. 4, lanes 2, 3 and 4) it is clear that P1 is produced in the same order of magnitude as polyhedrin protein. Northern blot analysis of transcripts indicated that the SV40 termination signals did not result in an absolute stop of transcription (P. W. Roelvink, personal communication), but this is also the case for SV40 transcription.

Recently, Vialard *et al.* (1990) have described an alternative polyhedrin-based baculovirus vector using β -galactosidase as a marker for screening for recombinant viruses. In this vector, *lacZ* expression is driven by a duplicated p10 promoter located about 900 nt upstream of the polyhedrin promoter and inserted in the opposite orientation with respect to transcription. The polyhedrin promoter remains available for expression of foreign

genes. However, because this type of vector uses an additional strong baculovirus promoter, this may reduce the amount of recombinant protein produced as a consequence of competition for transcription or translation factors. These complications are not likely to occur with our expression vector. Under the control of a non-virus promoter (hsp70), *lacZ* was constitutively expressed in small amounts, whereas the foreign gene, in our studies P1 of CaMV, was expressed under the control of the polyhedrin promoter late in infection at high levels. To avoid the problem of overlapping opposite transcripts, SV40 termination sequences were inserted at the 3' end of the *lacZ* gene. This part of SV40 DNA (132 nt) contains polyadenylation sequences in both orientations. Insertion of these sequences in the expression vector resulted in an overlap of 30 nt of the opposite transcripts which has no consequence for P1 production (Fig. 4a, lanes 4 and 3, respectively).

The strategy of producing heterologous proteins by the polyhedrin-based expression system is mainly applicable to insect cell cultures. Recombinant baculoviruses lacking the polyhedrin gene do not infect insects very well because the non-occluded viruses are degraded in the insect gut upon ingestion. The availability of the hsp70-*lacZ* gene cassette makes it possible to retrieve recombinant viruses that express foreign genes under the control of the p10 promoter in the p10 locus, leaving the polyhedrin gene intact (Vlak *et al.*, 1990).

Insertion of the hsp70-*lacZ* gene cassette can also be used to explore the baculovirus genome in order to identify genes that are not essential for virus multiplication. Viable viruses will only be obtained when genes interrupted by the *lacZ* gene cassette are not necessary for virus replication.

CaMV P1 is the first plant virus protein to be expressed in insect cells using a baculovirus vector. The CaMV insert was faithfully transcribed and translated to produce a 46K protein, equivalent to a CaMV protein found in virus-infected turnip tissue (Fig. 4). It is not yet known whether CaMV P1 is post-translationally modified in this system, as is the case for many proteins of animal origin (Luckow & Summers, 1988). The expressed 46K protein reacts with specific anti-P1 serum raised against a β -galactosidase-P1 fusion protein produced in *E. coli* (Fig. 4). Antiserum raised against CaMV P1 made in insect cells reacts with CaMV-infected plant extracts in immunoblot analysis with a much lower background than antiserum to P1 made in bacteria. The availability of large amounts of authentic plant virus protein from insect cells provides the possibility of raising polyclonal, monospecific antiserum for diagnostic purposes and demonstrates another application of the baculovirus expression vector system.

CaMV P1 protein was detected in the cytoplasm of

AcAS1-infected insect cells in the form of long hollow fibre-like structures. It is not known whether these structures are also formed in CaMV-infected plants. The low background of gold particles throughout the cytoplasm of AcAS1-infected insect cells (Fig. 5c) is probably due to either a diffused presence of P1 in the cytoplasm or an antibody reaction with β -galactosidase.

The availability of large amounts of P1 and mono-specific, polyclonal antibodies should allow more detailed studies of the ultrastructure of the fibre-like structure and function of P1 in CaMV-infected plants.

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