Foreign gene expression by a baculovirus vector with an expanded host range

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A nuclear polyhedrosis virus (NPV) (Baculoviridae)-based gene expression system was improved by DNA recombination. The BmN cell line established from Bombyx mori and the Sf21 cell line established from Spodoptera frugiperda are non-permissive for Autographa californica multicapsid NPV (AcMNPV) and B. mori NPV (BmNPV) replication, respectively. After cotransfection of AcMNPV DNA and BamHI-digested BmNPV DNA into Sf21 cells, progeny viruses were isolated by plaque purification on BmN cell monolayers and the host specificity of one viral isolate was analysed. The virus had a wider host range, and replicated and produced polyhedra in Sf21 cells, BmN cells and larvae of the silkworm, B. mori. DNA restriction endonuclease analysis showed that the isolate was a hybrid of AcMNPV and BmNPV. Using the AcMNPV transfer vector pAcYM1 a portion of the polyhedrin gene of the hybrid virus was replaced with the coding region of the firefly luciferase gene, producing a recombinant virus. The latter expressed firefly luciferase in both cell lines and in silkworm larvae under the control of the polyhedrin promoter.

Nuclear polyhedrosis viruses (NPVs) have genomes of approximately 130 kb of double-stranded, circular DNA and are members of the subfamily Eubaculovirinae (family Baculoviridae) (Francki et al., 1991). At a very late stage of infection, virus particles are embedded within a protein capsule (polyhedron) composed primarily of a single protein known as polyhedrin, which constitutes 20% or more of the total protein of the infected cells and is not essential for virus replication. Baculovirus expression vector systems take advantage of the strong polyhedrin promoter to drive the expression of foreign genes (Summers & Smith, 1987; Luckow & Summers, 1988; Maeda, 1989).

The system utilizing Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) and Spodoptera frugiperda cells is widely used for the production of many heterologous proteins, owing to the ease of producing large scale cultures of S. frugiperda cells in serum-free medium. Another baculovirus gene expression system, utilizing Bombyx mori NPV (BmNPV), can take advantage of the inexpensive high level production of heterologous proteins in B. mori (silkworm) larvae. These two baculovirus gene expression systems offer unique advantages.

Baculoviruses cause disease in larval insects and generally have a narrow host specificity. These properties make baculoviruses potentially very useful biological agents for the control of insect pests. To increase their usefulness as effective biological insecticides, it is desirable to alter these viruses genetically to be more virulent and to infect a broader host range (Roosien et al., 1986). AcMNPV infects 28 insect species (van der Beek, 1980) and replicates in seven lepidopteran cell lines (Knudson, 1980). However, this virus does not replicate and form polyhedra in the B. mori cell line (Summers et al., 1978), which is susceptible to BmNPV infection.

Heterologous recombinations between AcMNPV and BmNPV have been reported (Kondo & Maeda, 1991). After coinfection of BmNPV and AcMNPV into various lepidopteran cell lines, progeny viruses with a wider host range phenotype were isolated. It was suggested that the mechanism of host range expansion of these viruses was due to (i) their recombinant nature and (ii) a helper effect on viral replication. In the present study we also isolated host range-expanded NPVs for use as an improved baculovirus gene expression vector capable of utilizing S. frugiperda cells, B. mori cells and silkworm larvae. We demonstrate that this virus with a wider host range could be successfully employed for the production of large amounts of a foreign gene product, such as firefly luciferase.

The S. frugiperda cell line IPLB-Sf-21-AE (Sf21) and the B. mori cell line BmN were used and maintained in tissue culture flasks in TC-10 medium containing 10% foetal calf serum (FCS) (Brown & Faulkner, 1977).
AcMNPV was kindly supplied by Professor David Bishop (NERC Institute of Virology and Environmental Microbiology, Oxford, U.K.) and BmNPV was isolated in our laboratory by plaque purification. AcMNPV and BmNPV genomic DNA was extracted and purified from viral particles according to the method of Summers & Smith (1987).

To isolate host range-expanded NPVs, Sf21 cells were transfected with 1 μg of AcMNPV genomic DNA and 10 μg of BmNPV genomic DNA digested with BamHI using a CellPhect Transfection Kit (Pharmacia). After incubation at 27 °C for 5 days, the supernatant fluid was harvested. The transfection supernatant was diluted and subjected to plaque assay using BmN cell monolayers to isolate viruses with wider host specificity. Plaques exhibiting many polyhedra, as determined by light microscopy after 3 to 4 days of incubation, were picked and suspended in 1 ml of medium. Following three rounds of plaque purification using Sf21 and BmN cells, one virus isolate with a wider host range was purified. This isolate replicated in Sf21 and BmN cells and produced many polyhedra in the nuclei of both infected cell lines.

When the restriction enzyme digestion pattern of the DNA of this virus was compared with the parental AcMNPV and BmNPV genomic DNA patterns, the host range-expanded virus was nearly identical to AcMNPV whereas only a few fragments were shared with BmNPV. This indicated that the host range-expanded NPV is a hybrid virus (HyNPV) of AcMNPV and BmNPV (Fig. 1).

The origin of the polyhedrin gene in HyNPV was investigated by Southern blot hybridization. Viral DNAs of AcMNPV, HyNPV and BmNPV digested with EcoRI were hybridized with the AcMNPV EcoRI I fragment containing the polyhedrin gene (Hooft van Iddekinge et al., 1983). The probe hybridized to comigrating fragments of EcoRI-digested AcMNPV and HyNPV genomic DNAs, whereas the hybridization pattern of BmNPV genomic DNA was different (Fig. 2). The polyhedrin genes of AcMNPV and BmNPV contain a restriction site for KpnI and XbaI, respectively (Hooft van Iddekinge et al., 1983; Iatrou et al., 1985). Viral DNA fragments generated following digestions with EcoRI plus KpnI and EcoRI plus XbaI were hybridized with the AcMNPV EcoRI I fragment. The hybridization profiles showed the presence of the KpnI site and absence of the XbaI site in the polyhedrin gene of HyNPV (Fig. 2). These results indicated that the polyhedrin gene of HyNPV was derived from AcMNPV genomic DNA.

The strategy for construction of the recombinant transfer vector pAcLuc is shown in Fig. 3. The luciferase gene of the firefly, Photinus pyralis, was excised by digestion with HindIII and SmaI from the plasmid pSVOA/L (de Wet et al., 1987), treated with the Klenow fragment of DNA polymerase I, and ligated into the dephosphorylated SmaI site of the pAcYM1 transfer vector (Matsuura et al., 1989) to construct the recombin-
Fig. 3. Construction of baculovirus transfer vector pAcLuc. The luciferase gene coding region was excised with HindIII and Smal, filled in with the Klenow fragment of DNA polymerase I and ligated into the Smal site of the pAcYM1 polylinker.

ant transfer vector pAcLuc. The correct transcriptional orientation was determined using restriction enzyme mapping with EcoRV. BmN cells were transfected with 1 μg of viral DNA from HyNPV and 25 μg of pAcLuc DNA using the CellPhect Transfection Kit. After incubation at 27 °C for 5 days, the supernatant fluid was harvested, diluted, and subjected to plaque assay using Sf21 cell monolayers. Recombinant viruses were selected by light microscopy for polyhedron-negative plaques. Pure recombinant virus was isolated by three rounds of plaque assay and propagated on a Sf21 cell monolayer to produce stock virus with a titre of 1 x 10⁷ p.f.u./ml.

To examine the expression of the firefly luciferase in recombinant HyNPV-infected insect cells and silkworm larvae, luciferase activity was assayed using a luminometer. Sf21 and BmN cells (1 x 10⁶ cells per 35 mm plate) were inoculated with the luciferase gene-containing recombinant virus at an m.o.i. of 20 p.f.u. per cell. The virus was allowed to adsorb for 1 h at room temperature. The inoculum was removed and replaced with 2 ml of TC-10 containing 10% FCS. After incubation at 27 °C for 3 days, infected cells were scraped from the plate and pelleted by centrifugation at 4000 g for 10 min. After the supernatant had been discarded, the cell pellet was washed with TBS (10 mM-Tris–HCl pH 7.2, 130 mM-NaCl), resuspended in 200 μl of TBS, and disrupted in a Teflon homogenizer. Fifth instar larvae of B. mori were infected with a viral inoculum containing 5 x 10⁵ p.f.u. Haemolymph and fat bodies were collected from larvae at 4, 5 and 6 days post-infection. One microgram of fat body was suspended in 10 μl of TBS and disrupted in a Teflon homogenizer. These crude enzyme samples (1 μl) were added to 800 μl of 25 mM-glycylglycine buffer pH 7.5 containing 5 mM-ATP and 15 mM-MgSO₄ in a small test-tube. The tube was placed in a luminometer equipped with a chart recorder, and the reaction was initiated by injection of 200 μl of 1 mM-luciferin into the reaction cocktail. When the amount of expressed luciferase was calculated from comparison with light units of commercial purified luciferase (Sigma), the 1 μl samples of the homogenates of the recombinant virus-infected Sf21 and BmN cells were found to contain 6-0 x 10⁴ and 1-2 x 10⁵ light units, respectively. Light emission was not detected in HyNPV-infected or uninfected cells. Luciferase activity was also detected in the haemolymph and fat bodies of the recombinant virus-infected larvae, and increased after prolonged infection (Table 1).

A hybrid baculovirus derived from AcMNPV and BmNPV thus has been developed for use as an improved foreign gene expression vector for synthesis of heterologous proteins. The advantages of this vector include (i) ease of maintenance in vitro of the host cells (S.frugiperda cells), (ii) ease of transfection and plaque purification using these cells and (iii) ease of utilization of an in vivo host, silkworm larvae, which can efficiently express large amounts of heterologous protein.

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Table 1. Firefly luciferase activity in recombinant baculovirus-infected larvae

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<tr>
<th>Sample</th>
<th>Time after infection (days)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Haemolymph</td>
<td>ND*</td>
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<tr>
<td>Fat body</td>
<td>ND</td>
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* ND, Not detected.
† The values represent light units (x 10⁻⁴) of firefly luciferase activity.

References


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