Expression of *Bombyx mori* cytoplasmic polyhedrosis virus polyhedrin in insect cells by using a baculovirus expression vector, and its assembly into polyhedra

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A cDNA encoding the cytoplasmic polyhedrin of *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV) strain H was introduced into an improved baculovirus expression vector which can be utilized to express foreign genes in the *Spodoptera frugiperda* cell line IPLB-SF-21AE (Sf21 cells) and the *B. mori* cell line BmN. A recombinant virus produced large, cubic inclusion body-like structures in infected Sf21 and BmN cells. Western blot analysis showed that these structures were BmCPV polyhedra. This result suggested that the supramolecular assembly of BmCPV polyhedrin is responsible for its properties.

Cytoplasmic polyhedrosis viruses (CPVs) belong to the family Reoviridae based on virus morphology and the presence of a segmented dsRNA genome (Matthews, 1982). Virus infection is characterized by the formation of large proteinaceous inclusion bodies called polyhedra in the cytoplasm of the infected cells. The polyhedra are formed by the assembly of the viral polyhedrin polypeptide, of Mr 30K (Mori & Kawase, 1983), and occlude many virus particles. The cytoplasmic polyhedrin genes of *Bombyx mori* CPV (BmCPV) strains H and A (Arella et al., 1988; Mori et al., 1989) and *Euxoa scandens* CPV (EsCPV) (Fossiez et al., 1989) have been cloned and sequenced. Fossiez et al. (1989) reported that although the nucleotide sequence of EsCPV polyhedrin gene and the predicted amino acid sequence of the protein show no homology with those of BmCPV, the hydrophilicity profiles and predicted secondary structures resemble each other, especially in the N-terminal half of the cytoplasmic polyhedrin.

Nuclear polyhedrosis viruses (NPVs), members of the family Baculoviridae, also produce proteinaceous inclusion bodies. The infectious virus particles are embedded within a macromolecular paracrystalline protein matrix, which is composed of a single virus-encoded protein, polyhedrin (reviewed by Rohrmann, 1986). Jarvis et al. (1991) have recently determined the domain necessary for the supramolecular assembly of baculovirus polyhedrin into occlusion-like particles. However, the mechanism of the supramolecular assembly of CPV polyhedrin has not been elucidated.

Baculovirus gene expression vectors have become an important eukaryotic expression system because the potential level of production of the foreign gene product is 20% or more of the total protein of the infected cell (Summers & Smith, 1987; Maeda, 1989; Luckow, 1991). Many virus-derived genes have been expressed in insect cells (Emery, 1991), and recombinant baculoviruses containing the p55 gag precursor gene (Gheysen et al., 1989) or the entire polio virus genome, except the 5' non-coding region (Urakawa et al., 1989), have been shown to produce virus-like particles in infected cells. Thus the baculovirus gene expression vector system can be used for studies of the mechanism of assembly of viral proteins. To understand the mechanism of the supramolecular assembly of CPV polyhedrin, the polyhedrin gene of BmCPV strain H was expressed by using a baculovirus gene expression system.

A cloned cDNA encoding the cytoplasmic polyhedrin gene of BmCPV strain H (Mori et al., 1989) was digested with *SnaBI* and *BamHI*, and the *BamHI* site was repaired by using the Klenow fragment of DNA polymerase I. The cDNA fragment was ligated into the calf intestine alkaline phosphatase-treated *SmaI* site of the pAcYM1 baculovirus transfer vector (Matsuura et al., 1989), to construct the recombinant transfer vector pAcCP-H (Fig. 1). Restriction enzyme analysis using *EcoRV* and DNA sequencing was used to confirm that the coding sequence of the BmCPV polyhedrin gene was correctly oriented with the baculovirus polyhedrin promoter. BmCPV polyhedrin was expressed from an improved baculovirus expression vector derived from a host range-expanded baculovirus, which is a hybrid NPV...
(HyNPV) between *Autographa californica* multi-nucleocapsid NPV and *BmNPV* (Mori *et al.*, 1992). This virus can be utilized for foreign gene expression in the *Spodoptera frugiperda* cell line IPLB-SF-21AE (Sf21 cells), the *B. mori* cell line BmN and silkworm larvae (Mori *et al.*, 1992). Sf21 cells seeded in 35 mm Petri dishes were cotransfected with 1 pg of HyNPV DNA and 25 gg pAcCP-H plasmid DNA by the calcium phosphate technique using a CellPhect Transfection Kit (Pharmacia). The culture fluid was harvested after 5 days and subjected to plaque assay (Brown & Faulkner, 1977).

A plaque containing large, cubic inclusion body-like structures was selected using inverted phase-contrast microscopy, and virus was purified in three consecutive plaque assays and propagated on an Sf21 cell monolayer to produce a stock with a titre of 1 × 10⁷ p.f.u./ml. Sf21 and BmN cells were infected at a multiplicity of about 5 p.f.u./cell and incubated at 27 °C for 3 days. Many inclusion body-like structures were produced in the recombinant virus-infected cells (Fig. 2). The structures produced in Sf21 cells were larger than those in BmN cells and localized to the periplasm and the centre of the infected cell.

The inclusion body-like structures were purified from infected Sf21 cells as described previously (Mori & Kawase, 1983) and dissolved in sample buffer (Laemmli, 1970) by heating at 100 °C for 5 min. The dissolved proteins were subjected to 12.5 % SDS-PAGE and transferred electrophoretically onto a nitrocellulose membrane. The membrane was saturated with washing buffer (20 mM-Tris-HCL pH 7.5, 500 mM-NaCl) containing 1 % gelatin, and incubated overnight at 25 °C with a 1:3000 dilution of mouse immune serum directed against the BmCPV polyhedrin. After several washes, the membrane was incubated successively for 1 h at 25 °C with a 1:3000 dilution of goat anti-mouse IgG conjugated with horseradish peroxidase (Bio-Rad). After washing, the bound antibody was detected by using a Konica Immunostaining HRP kit. Analysis of the proteins derived from the inclusion body-like structures by SDS-PAGE showed a single band with M₀, 30K, and Western blot analysis showed that the proteins reacted with an antibody directed against the BmCPV polyhedrin. After several washes, the membrane was incubated successively for 1 h at 25 °C with a 1:3000 dilution of goat anti-mouse IgG conjugated with horseradish peroxidase (Bio-Rad). After washing, the bound antibody was detected by using a Konica Immunostaining HRP kit. Analysis of the proteins derived from the inclusion body-like structures by SDS-PAGE showed a single band with M₀, 30K, and Western blot analysis showed that the proteins reacted with an antibody directed against the BmCPV polyhedrin. These results indicated that the inclusion body-like structures were BmCPV polyhedra (Fig. 3). No BmCPV particles or other BmCPV proteins were present in cells infected with the recombinant baculovirus. This indicated that BmCPV polyhedrin possesses the properties for supramolecular assembly and that the assembly of BmCPV polyhedrin occurs by itself.
Fig. 3. SDS-PAGE and Western blot analysis of BmCPV polyhedrin produced by a recombinant baculovirus. Proteins from inclusion body-like structures (lanes 1) and cell lysates of Sf21 (lanes 2, 3 and 4) and BmN (lanes 5, 6 and 7) were subjected to (a) 12.5% SDS-PAGE and stained with Coomassie blue, or (b) Western blotting with a polyclonal mouse antiserum raised against BmCPV polyhedrin. Lanes 2 and 5, mock-infected cell lysate; lanes 3 and 6, HyNPV-infected cell lysate; lanes 4 and 7, recombinant virus-infected cell lysate. M, are shown to the left.

Sf21 and BmN cells were seeded at 1 x 10^6 cells/dish, infected at a multiplicity of about 5 p.f.u./cell and incubated for 3 days at 27°C. Infected cells were harvested, washed with ice-cold TNM buffer (10 mM-Tris·HCl pH 7.0, 140 mM-NaCl, 3 mM-MgCl₂) and resuspended in 200 μl of TNM buffer. The suspensions were mixed with an equal volume of 2× sample buffer and subjected to Western blot analysis as described above. The amount of CPV polyhedrin in the recombinant virus-infected Sf21 cells was greater than that in BmN cells (Fig. 3). CPV polyhedra in recombinant virus-infected Sf21 and BmN cells were cubic, and the solid structure was very similar to that of BmCPV strain H polyhedra (Fig. 4). The sizes of polyhedra in the Sf21 and BmN cells were 2.42±0.85 and 1.64±0.61 μm along the edge, respectively (Fig. 4). These results suggest that the size of CPV polyhedra is dependent on the amount of CPV polyhedrin in the insect cell.

The intracellular distribution of CPV polyhedra was studied by cell fractionation. The culture medium of recombinant virus-infected Sf21 cells was removed, and loose cells were washed with ice-cold TNM buffer. To disrupt the cytoplasmic membrane and separate CPV polyhedra in the cytoplasm from nuclei containing CPV polyhedra, solubilizing buffer (10 mM-Tris·HCl pH 7.0, 1% v/v NP40) was added to the dishes. The dishes were scraped with a rubber policeman and the extract was harvested and centrifuged for 5 min in a microcentrifuge. The pellet was resuspended in TNM buffer, and the

Fig. 4. Scanning electron micrographs of inclusion body-like structures in recombinant virus-infected Sf21 (a) and BmN (b) cells. Bar markers represent 5 μm.
Currently investigating the domain necessary for the nucleus and the cytoplasm is not known. We are well characterized nuclear localization signals, that nucleus of recombinant virus-infected cells. Jarvis mechanism of intracellular localization of BmCPV cytosolic fraction. These two fractions were collected and polyhedra in the cytoplasm were pelleted and used as the derived from the simian virus 40 (SV40) T antigen has been studied in great detail. It consists of a proline for nuclear localization of NPV polyhedrin. Among the Butel, 1984). However, there is no tract of basic residues BmCPV polyhedra localized in the cytoplasm and the subject to Western blot analysis. As shown in Fig. 5, formed a visible band at the interface between 1.05 and 1.13 g/ml and were used as the nuclear fraction. CPV polyhedra of the silkworm, Bombyx mori, contained CPV polyhedra localized in the cytoplasm and the nucleus of recombinant virus-infected cells. Jarvis et al. (1991) have reported that the sequence KRKK is essential for nuclear localization of NPV polyhedrin. Among the well characterized nuclear localization signals, that derived from the simian virus 40 (SV40) T antigen has been studied in great detail. It consists of a proline residue followed by a stretch of basic residues, PKKKRKV (Kalderon et al., 1984a, b; Lanford & Butel, 1984). However, there is no tract of basic residues similar to this signal in BmCPV polyhedrin (Mori et al., 1989), and the reason BmCPV are produced in the nucleus and the cytoplasm is not known. We are currently investigating the domain necessary for the supramolecular assembly of CPV polyhedrin, and the mechanism of intracellular localization of BmCPV polyhedrin.

References


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