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

Hajime Mori,1* Reiko Ito,1 Hiroshi Nakazawa,1 Motoyuki Sumida,1 Fujiyoshi Matsubara1 and Yuzo Minobe2

1 Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto 606 and 2 National Institute of Agrobiological Resources, Tsukuba 305, Japan

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 M, 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 poliovirus 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 *SmaBI* 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
Short communication

Fig. 1. Schematic diagram of the construction of transfer vector pAcCP-H. The full-length BmCPV polyhedrin gene was excised from plasmid pBRH by SnaBI and BamHI digestion, and ligated to the SmaI site of the pAcYM1 vector as described in the text. The sequence of the 5' insertion site was determined by the methods of Sanger et al. (1977).

Fig. 2. Micrographs of recombinant virus-infected Sf21 (a, b and c) and BmN (d, e and f) cells. Cells were infected at a multiplicity of 5 (or mock-infected). Mock-infected cells (a, d), HyNPV-infected cells (b, e) and recombinant virus-infected cells (c, f).

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 transblotted electrophoretically unto 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 M., 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.
Sf21 and BmN cells were seeded at $1 \times 10^6$ 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-MgCl$_2$) 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 $2.42 \pm 0.85$ and $1.64 \pm 0.61$ 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. 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. Mr's are shown to the left.

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.
suspension was layered on a Percoll (Pharmacia) step gradient (density 1.05 and 1.13 g/ml) in a microcentrifuge tube and centrifuged at 5000 r.p.m. in a microcentrifuge for 30 min at 4 °C. The nuclei containing CPV polyhedra formed a visible band at the interface between 1.05 and 1.13 g/ml and were used as the nuclear fraction. CPV polyhedra in the cytoplasm were pelleted and used as the cytosolic fraction. These two fractions were collected and subjected to Western blot analysis. M's are shown to the left.

Fig. 5. Subcellular distribution of the BmCPV polyhedrin. BmCPV polyhedrin in recombinant virus-infected Sf21 cells was fractionated into cytosolic (C) and nuclear (N) fractions. Samples from each fraction were subjected to Western blot analysis. M's are shown to the left.

References