Expression of animal virus genes using Baculovirus AcNPV

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Out of the many systems employed for the expression of foreign genes, bacterial systems are by far the simplest and most efficient ones. They are relatively cheap and often give high protein yields, however, they have some drawbacks: in bacteria RNA is processed in a way different than in Eukaryota, and the post-translational modifications of proteins are much less pronounced, e.g. bacteria cannot glycosylate polypeptide chains. Therefore although eukaryotic systems are less efficient than those of bacterial origin, the foreign gene expression based on yeast or mammalian cells yields recombinant proteins more similar to their original counterparts. The system based on an insect virus, Baculovirus, while retaining most of the advantages of eukaryotic systems (for foreign gene expression), gives high yields of recombinant proteins at relatively low cost.

Baculoviruses are viral pathogens that cause a fatal disease (polyhedrosis) in insects, mainly in the members of Lepidoptera and Diptera. In nature, the virus enters the larvae via ingested food, it is released from its envelope in the midgut and then infects the midgut cells. Progeny viruses are released to hemolymph and they become transported to other organs. Viral particles, produced at the later stages of infection, are embedded in a protein envelope, composed mainly of a single protein called polyhedrin. Synthesis of this compound starts 24 h after the infection and continues until ultimate cell lysis. In a cell culture polyhedrin is not essential component because polyhedrin is necessary to protect viral particles in the natural environment only. The promoter of the polyhedrin gene is very potent (1 mg of polyhe-

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1 Abbreviations: NP gene, nucleoprotein gene; AcNPV, Autographa californica Nuclear Polyhedrosis Virus
Fig. 1. The transplacement plasmids pVL 1392 and pVL 1393.
A. Plasmids were based on pUC 8 (V), contain AcNPV DNA flanking a multicloning site for foreign gene insertion, (P) promoter region of polyhedrin gene and flanking sequence, (T) terminator region of polyhedrin gene and flanking sequence, (MCS) multicloning sites. The flanking AcNPV sequences facilitate allelic replacement of the polyhedrin gene of wild type virus with the foreign gene to yield recombinant virus vectors. B. Multicloning sites for vectors pVL 1392 and pVL 1393.
Fig. 2. Polyacrylamide gel-SDS electrophoresis of the NP protein of influenza virus produced in baculovirus. Full length NP is marked with an arrow. 1, Molecular weight markers; 2-8, nuclear fractions of Sf9 cells infected with recombinant baculovirus (1-7 days post infection) and 43, cytoplasmic fractions of infected cells (1-8 days post infection).

The second gene cloned to baculovirus transfer vectors was the gene coding for the gI glycoprotein of pseudorabies virus. This glycoprotein is one of the envelope proteins responsible for virulence in pigs and it is one of the candidates for a subunit vaccine. Genomic DNA of Pseudorabies virus (150 kb) was digested with BamHI and the 7 kb fragment containing a few genes including gI gene was cloned into high copy plasmid pIT7-5. Subsequently, the 2.4 kb fragment containing only gI gene was subcloned into EcoRI/BamHI sites of the transfer vector pVL 1392. The recombinant baculoviruses were detected using a probe prepared by labelling the DNA segment encompassing the gI gene with digoxigenin. Our preliminary results indicate that the level of the recombinant protein is in this case lower than that for the NP polypeptide and we are going to attempt optimization of the conditions for its expression.

REFERENCES