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THE GENETIC ENGINEERING OF BACULOVIRUS FOR FOREIGN GENE EXPRESSION

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There are several unique properties of baculoviruses which allow them to be genetically engineered so that they can be used as foreign gene expression vectors; that is, genes from other viruses, plants, animals or microbes can be inserted into one baculovirus genome and high levels or protein of that inserted gene can be expressed. The first property that makes the baculoviruses unique is that two forms of the virus are produced. The extracellular virus or nonoccluded virus is produced early in the infection process. Later during infection from 18 hours the second form of the virus, the occluded virus or polyhedra, begins to form in the nucleus. This form of the baculovirus is characterized by enveloped nucleocapsids that are surrounded by a paracrystalline protein matrix called polyhedrin. There can be several to about 100 polyhedra per infected cell. This can represent 25 to 50% of the cell mass.

Infected cell protein synthesis has shown that the extracellular virus proteins are complete by 10 to 15 hours post infection. The polyhedrin protein was beginning to be synthesized between 15 to 24 hours post infection. At 24 hours post infection the polyhedrin protein is the major protein synthesized (Maruniak & Summers, 1981). It has been shown that baculovirus proteins can be both phosphorylated or glycosylated with mannose, N-acetyl-glucosamine or N-acetyl-galactosamine (Maruniak, 1979).

Baculoviruses replicate in cell cultures and plaque assays can be done (Knudson, 1979). One other important feature is that baculovirus DNA is infectious; it can be transfected into cells to produce plaques (Burand et al., 1980).

Taking this together, baculoviruses have several features that make them candidates for genetic engineering: two viral forms, ability to replicate in cell cultures, infectious DNA, the ability to glycosylate or phosphorylate proteins, and they have a late gene product produced in abundant quantities.

It was necessary to determine whether the AcNPV polyhedrin gene was essential since this was a promising gene for genetic engineering. First the AcNPV polyhedrin gene was mapped to the genome (Vlak et al., 1981) and the DNA sequenced (Hooft van Iddekinge et al., 1983). Site-directed mutagenesis has been a powerful technique for determining the functional and necessity of a gene. This was done to the AcNPV EcoR1-I restriction fragment containing the gene for polyhedrin and flanking DNA. The cloned EcoR1-I fragment was cut with Kpn1 which is a unique restriction site within the polyhedrin gene. The ends of the DNA were digested with Exonuclease III or S1 nuclease. This shortened piece of DNA was repaired at the ends with DNA polymerase and ligated together with T4 DNA ligase. The cloned mutated polyhedrin gene was cotransfected with the wild type AcNPV DNA to be transferred back to the viral genome. Recombination occurred in the AcNPV DNA flanking the polyhedrin gene. Plaques were observed that did not produce polyhedra along with wild type polyhedra positive plaques. The polyhedra negative plaques were recombinants between the mutated gene and wild type DNA (Smith et al., 1983a). This proved that the late polyhedrin gene was not essential for extracellular virus replication. All of the criteria were in place to attempt to express a foreign gene.

Would the polyhedrin promoter (which was still intact) in the mutated transfer vector permit transcription and processing of foreign genes? The first publication to show that not only would it express the gene, but it would at high levels, was by Smith et al. (1983b). In this publication, they used the human beta-interferon gene and inserted it into several transfer vectors. One transfer vector had an intact ATG start codon and some polyhedrin coding sequences before the ATG start codon for the interferon

gene. The recombinant AcNPV genome with this gene construct produced high levels of interferon protein fused to some amino acids of polyhedrin, as well as nonfused interferon. If the interferon gene with an ATG start codon was inserted into a transfer vector without an ATG start codon but with the AcNPV promoter sequences upstream intact, the recombinant virus produced no fused protein and the highest levels of beta-interferon were produced and secreted from the infected insect cells. It was also shown that the interferon protein was glycosylated by the insect cells (Smith et al., 1983b).

Since this first report, numerous publications have demonstrated the usefulness of the baculovirus expression vectors to produce high levels of proteins from organisms as diverse as *Eschericia coli*, Drosophila, Hepatitis virus, AIDS virus, humans, fungi and plants. The gene inserts range from 700 to 9200 base pairs of DNA (reviewed by Luckow & Summers, 1988). Proteins that have been produced have been shown to have biological and serological activity. The insect cells appear to correctly cleave signal peptides. These recombinant proteins can also be glycosylated and phosphorylated in the insect cell cultures. To date, the baculovirus expression vector system has been shown by numerous scientists to produce the highest levels of recombinant proteins of any expression system. Since polypeptides are correctly processed, secreted and modified, this system is much more useful in general than prokaryotic expression systems. Because baculoviruses can be replicated in insect cell cultures or larvae, potentially large quantities of inexpensive proteins useful to medicine, research and agriculture will be produced.

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