THE POTENTIAL OF GENETICALLY ENGINEERED NUCLEAR POLYHEDROSIS VIRUSES IN THE CONTROL OF *HELIOTHIS*

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INTRODUCTION

The bollworm species *Heliothis armigera* and *H. punctigera* are major pests of cotton in Australia and their control is vitally important in cotton management programs. At present, populations of these species are controlled by the use of organic insecticides. However, repeated occurences of resistance to these chemicals in *H. armigera* indicate a need to develop alternative forms of control. One such form is biological control, which relies on the use of natural pathogens and parasites to control the pest species. A group of pathogens that have been used for the biological control of several insect pests are the nuclear polyhedrosis viruses (NPVs).

Five features of NPVs give them great potential for use as biological control agents:

(i) they are highly specific, e.g. *Heliothis* NPVs only infect *Heliothis* species, so application of the virus does not affect beneficial insects. Furthermore, NPVs are non-toxic to vertebrates, including man;

(ii) because NPVs, like other biological control agents, are generally maintained within a balanced host-parasite system, resistance is unlkely to evolve in the same manner as resistance to chemical insecticides;

(iii) insects resistant to chemical insecticides will still be susceptible to NPVs. Therefore, they are of use in populations in which chemical insecticide resistance has evolved;

(iv) NPVs persist in the environment on plant surfaces and in the soil, therefore, a single application of the virus can produce infections in subsequent generations;

(v) they leave no toxic residues in the environment.

A commercial formulation of a *Heliothis* NPV has previously been imported into Australia and marketed under the name of Elcar™. Originally integrated into the computer based management program, FLY (a precursor of SIRATAC - Hearn *et al.*, 1981), initial trials were encouraging and yields were maintained with fewer pesticide applications than in conventionally treated cotton crops (Room, 1979). However, although integrated into the SIRATAC program, inconsistent performance led to its removal as a pesticide option in 1980-81.

Three major factors are thought to have contributed to the inefficiency of Elcar™ in field situations. First, the virus has a relatively short half-life, 1-2 days when exposed to u/v irradiation - a feature common to all NPVs (Smith, 1976). Second, it has a relatively slow rate of kill (about 4-6 days), during which time infected larvae continue to feed and cause damage (Teakle *et al.*, 1985). Third, the virus is inefficient against medium to large sized larvae when they are treated at economically feasible levels (Teakle *et al.*, 1985).

The CSIRO Division of Entomology has recently received support from the Cotton and Oilseeds Research Councils to establish a research program to manipulate the genome of *H. armigera* NPV (HaNPV) *in vitro*. The overall aim of the program is to produce novel strains of HaNPV that have greater potential for the control of Heliothis. These aims will be achieved by genetically engineering viruses that have a faster rate of kill and are effective against large larvae, but retain the beneficial features of the wild-type virus.

GENETIC MANIPULATION OF NPVs

NPV virus particles are bacilliform (bacteria-like) in shape and contain a closed circle of double-stranded DNA. The virus particles are embedded in a large protein matrix called the polyhedral body, or polyhedron. The protein that forms this matrix, termed polyhedrin, is produced by a viral gene late in the virus' replicative cycle. Although the polyhedral body stabilises the

embedded virus particles, it is not ultimately necessary for the virus particles to be infectious. For this reason, and because the polyhedrin protein is produced in very large quantities, the polyhedrin gene has, to date, been the most widely used system for the genetic manipulation of NPVs.

Early in the 1980's Gale Smith and colleagues at Texas A&M University developed a technique for deleting the coding region of the polyhedrin gene of the *Autographa californica* NPV (AcNPV) and inserting foreign genes in its place (Smith *et al.*, 1983). When the virus containing the foreign gene is used to infect lepidopteran tissue culture cells, the foreign gene is expressed under the control of the polyhedrin gene's promoter. (The promoter lies next to the gene and contains the signals that switch it on and off, Figure 1).

Using systems similar to those developed by Smith *et al.* (1983), it has since proved possible to introduce many genes into the AcNPV genome and to have them expressed under the control of the polyhedrin promoter in tissue culture cells. Many of the genes introduced into NPVs to date are of biomedical value, for example insulin and human growth factor. In these cases the virus is being used primarily as an expression system for the mass production of these proteins (Lucknow and Summers, 1988). Recently the technique has been developed one step further and a modified virus used to infect whole insects (in this case the silkworm, *Bombyx mori*); good levels of production were still achieved from the introduced gene (Marumoto *et al.*, 1987).

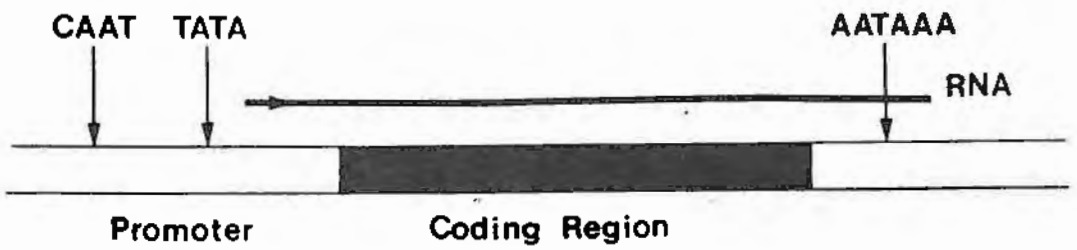

Figure 1. Schematic representation of the AcNPV polyhedrin gene and its control elements. The CAAT & TATA sequences in the promoter region (approx. 100 b.p.), control the transcription of RNA. The AATAAA signal is necessary for the RNA to be polyadenylated and transported into the cytoplasm where it is translated into protein, i.e. polyhedrin.

With the latter development, it therefore becomes possible to introduce a foreign gene into the genome of an NPV which will increase its efficiency as a biological control agent. The type of genes that are being considered at the present moment include toxin genes, hormone genes, neuropeptide genes and hormone modifying genes. These genes produce proteins which, like the NPVs into which they are being inserted, are insect specific. The engineered viruses produced will therefore have the same host range as the wild-type virus and leave no lasting residues in the environment.

Although genetically engineered viruses are an attractive proposition as biological control agents, until recently one major problem has remained. In utilising the polyhedrin promoter, the engineered viruses are left unable to produce a polyhedral body. This is no disadvantage when the engineered NPV is grown in tissue culture, where polyhedral protection is unnecessary. However, such stability is crucial for the efficiency of the virus in field situations as a biological control agent.

Recently a group at the Institute of Virology in Oxford led by Prof. David Bishop has overcome this problem. They have demonstrated that a second copy of the polyhedrin promoter can be inserted into the NPV genome alongside a foreign gene. The foreign gene is expressed in the same fashion as the polyhedrin gene itself, and the engineered virus is produced in its polyhedral form (D.H.L. Bishop, *pers comm*).

MODIFICATION OF HaNPV

The work currently underway at the CSIRO Division of Entomology is aimed at adapting the technologies developed by the Texas and Oxford groups to HaNPV. The eventual aim is to insert a foreign gene into HaNPV which will overcome the problems of slow kill rate and ineffectiveness against medium and large sized larvae originally associated with Elcar™.

The HaNPV strain being used was originally isolated from *H. armigera* from S.E. Queensland (a gift from Dr R.E. Teakle). We are currently identifying and isolating the polyhedrin gene and promoter from this isolate. Projects currently underway elsewhere in the Molecular Biology Section of the Division are working to isolate and characterise suitable foreign genes for insertion into the HaNPV genome.

REFERENCES

Hearn, A.R., Ives, P.M., Thompson, N.J. and Wilson, L.T. (1981). Computer-based cotton pest management in Australia. Field Crops Res. 4, 321-332.

Lucknow, V.A. and Summers, M.D. (1988). Trends in the development of baculovirus expression vectors. Biotechnology 6, 47-55

Marumoto, Y., Sato, Y., Fujiwara, H., Sakano, K., Saeki, Y., Agata, M., Furosawa, M. and Maeda, S. (1987). Hyperproduction of polyhedrin-IGF II fusion protein in silkworm larvae infected with recombinant *Bombyx mori* nuclear polyhedrosis virus. J. Gen. Virol. 68, 2599-2606.

Room, P.M. (1979). A protoype "on-line" system for the management of cotton pests in the Namoi Valley, New South Wales. Protection Ecology 1, 245-264.

Smith, G.E., Summers, M.D. and Fraser, M.J. (1983). Production of human interferon in insect cells infected with a baculovirus expression vector. Mol. Cell Biol. 3, 2156-2165

Smith, K.M. (1976). Virus-Insect Relationships. Longman Press, London.

Teakle, R.E., Jensen, J.M. and Giles, J.E. (1985). Susceptibility of *Heliothis armiger* to a commercial nuclear polyhedrosis virus. J. Invertebr. Pathol. 46, 166-173.