The predicted amino acid sequence of the spheroidin protein from *Amsacta moorei* entomopoxvirus: lack of homology between major occlusion body proteins of different poxviruses

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Entomopoxviruses replicate in the cytoplasm of insect cells and characteristically produce occlusion bodies which serve to protect the virion from the environment; the major component of these bodies is a protein called spheroidin. We have previously identified and sequenced the gene encoding the major occlusion body protein of eastern spruce budworm (*Choristoneura biennis*) entomopoxvirus (CbEPV) and found it to encode a 47K polypeptide which aggregates due to the formation of intermolecular disulphide bonds. In this publication we demonstrate that the insect poxvirus of *Amsacta moorei* produces spheroidin with a unit Mr of 114.8K. The gene for this protein was cloned and sequenced, and the predicted polypeptide was demonstrated to contain 38 cysteine residues, a leucine zipper for possible protein-protein interactions and 14 potential Asn-linked glycosylation sites. Other than possessing a large number of sulphydryl groups, this protein showed no homology to its analogue found in cells infected with CbEPV. Antibodies directed against occlusion body proteins of the two viruses also failed to cross-react significantly on Western blots. In addition, nucleic acid probes prepared from the two different genes did not cross-hybridize on Southern blots of genomic DNA prepared from the viruses. Finally, the occlusion body proteins from the two insect viruses were compared with the A-type inclusion body protein of cowpox virus. Again, little homology between these proteins was evident, with the exception of a generally high cysteine content and a similarity between their late gene promoters. We conclude that the major occlusion body proteins of different poxviruses possess diverse primary structures, but all are capable of yielding large aggregates through the formation of disulphide bonds.

**Introduction**

Insect poxviruses, known as entomopoxviruses, have been isolated from at least 31 host species (Granados, 1981; Arif, 1984; Arif & Kurstak, 1991). These viruses resemble their mammalian counterparts both biochemically and morphologically (Pogo et al., 1971; McCarthy et al., 1975; Mathews, 1982), but they have a limited host range and will infect only the larvae from certain species of *Lepidoptera*, *Coleoptera*, *Diptera* and *Orthoptera*. Entomopoxviruses characteristically form occlusion bodies or spheroids which serve to protect the virions from the environment. Virus can be released from the protective bodies in the alkaline environment of the insect gut or under reducing conditions in the presence of sodium carbonate (McCarthy et al., 1974). Little is known about their molecular biology, but the entomopoxvirus of *Amsacta moorei* (AmEPV) is the most extensively studied member of the insect poxvirus family (Granados & Roberts, 1970; McCarthy et al., 1974, 1975; Langridge & Greenberg, 1981; Langridge et al., 1983; Langridge 1983a, b, c). The DNA genome of AmEPV is 222 to 242 kb in length and is rich in A + T residues (78 to 82%). Recently, the genome of this virus was purified by field inversion electrophoresis and the DNA was mapped by restriction endonuclease analysis (Hall & Hink, 1990). This particular entomopoxvirus can be propagated in cell lines derived from saltmarsh (*Estigmene acrea*), cabbage looper (*Trichoplusia ni*) and gypsy moth (*Lymantria dispar*) caterpillars (McCarthy et al., 1974; Langridge & Greenberg, 1981; Goodwin et al., 1990). Occlusion bodies are produced during infections...
in all three of these cell types. The major occlusion body protein (called spheroidin) of AmEPV has been characterized from *E. acrea* and *T. ni* cells, and found to have an *M*<sub>r</sub> of 110000 (Langridge, 1983a, b).

Eastern spruce budworm (*Choristoneura biennis*) is infected by another entomopoxvirus (CbEPV) and its molecular composition has also been studied (Arif, 1976; Bilimoria & Arif, 1979). Genomic DNA of this virus has been purified and determined to possess a high A+T content, 73.4 to 74.6%. Our laboratory recently cloned and sequenced the major occlusion body protein of CbEPV (Yuen *et al*., 1990). We found the protein to be rich in cysteine residues and to have a predicted *M*<sub>r</sub> of 38-5K. The post-translationally modified protein migrates on SDS–polyacrylamide gels as a 47K monomer which aggregates to form dimers and trimers in the absence of reducing agent. Amino-terminal sequencing of the protein demonstrated that CbEPV spheroidin possesses a signal peptide which is cleaved during maturation. The apparent difference in *M*<sub>r</sub>s between the spheroidin proteins of AmEPV and CbEPV prompted us to investigate the relationship between these two viruses more closely. In this report we have compared the major occlusion body proteins of CbEPV and AmEPV using SDS–PAGE and immunoblot techniques. In addition, DNA libraries were prepared from mRNA and genomic fragments, and the spheroidin gene of AmEPV was subsequently cloned and sequenced.

**Methods**

**Virus, insect cell lines and larvae.** Occlusion bodies for CbEPV and *C. fumiferana* entomopoxvirus (CfEPV) were supplied by the Forest Pest Management Institute, Forestry Canada (Sault Ste Marie, Ontario, Canada). Non-occluded and occluded AmEPV were a kind gift from Drs R. L. Hall and W. F. Hink (Department of Entomology, Ohio State University, Columbus, Ohio, U.S.A.). This virus originated from the laboratory of Dr R. R. Granados (Boyce Thompson Institute for Plant Research, Ithaca, New York, U.S.A.). Viruses were purified by plaque selection.

The BTI-EAA (*E. acrea*) haemocyte cell line also came from the laboratory of Dr R. R. Granados and was subcultured in our laboratory to give a homologous population which supported the growth of AmEPV. These cells were propagated in Grace's insect medium (Gibco) supplemented with 10% foetal calf serum (FCS). IPLB-LD-652 (*L. dispar*) cells were a gift from Dr M. Shapiro (Insect Pathology Laboratory, U.S. Department of Agriculture, Beltsville, Md., U.S.A.) and were cultivated in EX-CELL 400 medium (JRH Biosciences). The FPMI-CF16 cell line from *C. fumiferana* supported the growth of both CbEPV and CfEPV and was obtained from Dr Sardar Sohi (Forest Pest Management Institute, Sault Ste Marie, Ontario, Canada). *C. fumiferana* and *E. acrea* larvae were also supplied by the Forest Pest Management Institute.

**Purification of occlusion bodies from larvae.** CbEPV occlusion bodies were harvested from infected *C. fumiferana* larvae and AmEPV spheroids were obtained from infected *E. acrea* caterpillars. Larvae were infected by adding small quantities of the occlusion bodies to an artificial diet supplied by Bio-Serv. After death, the caterpillars were frozen at –20°C and lyophilized for 24 h. The dried insects (5 g) were subsequently ground in a mortar and pestle, and stirred in 100 ml water containing 0.5% SDS for 2 h. The suspension was subsequently filtered three times through multiple layers of cheesecloth. Filtrates were centrifuged at 1000 g for 15 min at room temperature, and the resulting pellet was resuspended in 100 ml distilled water and recentrifuged. The washing process was performed three times. Occlusion bodies were suspended in 20 ml of water, large particulate matter was allowed to settle for 5 min and the supernatant was placed on discontinuous sucrose gradients consisting of 10 ml 65% (w/w) sucrose, 12 ml 50% (w/w) sucrose and 3 ml 35% (w/w) sucrose in water. A sample (10 ml) was layered onto each gradient and the tubes were centrifuged at 25000 r.p.m. using a Beckman SW28 swinging bucket rotor. Purified occlusion bodies at the 65%/50% sucrose interface were aspirated, diluted with water and collected by centrifugation at 1000 g.

**Purification of occlusion bodies from an *E. acrea* cell line.** BTI-EAA cells were infected with non-occluded virus at a multiplicity of 1 to 5 p.f.u./cell. The infected cells were cultivated for 96 h in Grace's medium which contained 1% (v/v) rather than the usual 10% (v/v) FCS. Occlusion bodies were apparent by this time and 5 × 10<sup>6</sup> cells were harvested and collected by low speed centrifugation at 1000 g. The cell pellet was lysed in 50 ml of water containing 0.5% (w/v) SDS with a Dounce homogenizer containing a loose pestle. Occlusion bodies were collected by centrifugation at 1000 g for 15 min, resuspended in 10 ml of water and purified on the sucrose gradient described above.

**Southern blot analysis of entomopoxviruses DNA with probes constructed from the major occlusion body protein genes of CbEPV and AmEPV.** Genomic DNA was digested with restriction endonucleases *EcoRI, HindIII* or *XmnI*, or a combination of the three. DNA fragments were resolved on 0.7% agarose gels in the presence of ethidium bromide and transferred to nitrocellulose (Southern, 1975). Radioactive probes were constructed from the entire regions encoding the major occlusion body proteins using the Multiprime kit (Amersham). Filters were hybridized at 37°C as previously described (Vialard *et al*., 1990). After 24 h, the blots were washed four times with buffer composed of 2 × SSC and 0.1% SDS at 28 to 30°C, and exposed to film for 4 to 6 h.

**Purification of AmEPV DNA and construction of genomic libraries.** Genomic DNA was purified by suspending approximately 10<sup>10</sup> occlusion bodies and dissolving them in 2 ml of a buffer containing 0.8 M-sodium carbonate and 0.02 M-sodium thioglycollate for 1 h. The pH was lowered to pH 8.5 by adding dilute HCl, and DNA was released from virions by adjusting the solution to 1% SDS, 0.05 M-EDTA and 2 mg/ml proteinase K. The mixture was incubated at 65°C for 2 h, then diluted to 5 ml with water, and extracted with phenol and chloroform. Genomic DNA was precipitated with ethanol and sodium acetate, digested with *EcoRI, HindIII* or *XmnI*, or a combination of these enzymes, and ligated to the pUC19 cloning vector digested similarly.

**Purification of mRNA from infected *E. acrea* and *L. dispar* cells.** Messenger RNA from BTI-EAA and IPLB-LD-652 cells infected with AmEPV was prepared using an mRNA Purification Kit from Pharmacia LKB. Total RNA was extracted from 5 × 10<sup>6</sup> cells using guanidinium thiocyanate and RNA was sedimented by ultracentrifugation through caesium trifluoroacetate. Poly(A)<sup>+</sup> RNA was selected from total RNA using oligo(dT)-cellulose spin column chromatography.

**Synthesis of dsRNA from infected cell mRNA and construction of cDNA libraries.** A cDNA library was constructed from infected cell mRNA using the cDNA Synthesis Kit from Pharmacia LKB. Double-stranded DNA was synthesized from poly(A)<sup>+</sup> RNA using murine leukaemia virus reverse transcriptase and the Klenow fragment of DNA polymerase 1. *EcoRI/NotI* linkers were added to the dsRNA and the
product was ligated to the pUC19 vector which had been digested with EcoRI. Competent *Escherichia coli* DH5α was transformed with the ligated DNA.

**Electrophoretic purification and protein sequencing of AmEPV protein.** AmEPV occlusion bodies (10^9) were suspended in denaturation buffer consisting of 20 mM-DTT, 6 M-guanidine hydrochloride, 1 mg/ml EDTA and 100 mM-Tris–HCl pH 8.5, and were incubated at 37 °C for 2 h. Reduced disulphide groups were alkylated by adding 80 mM-iodoacetamide for a period of 0.5 h. Excess alkylating agent was subsequently allowed to react with 200 mM-DDT. The sample was dialysed three times against 100 mM-Tris–HCl pH 8.5 at 4 °C. Reduced and alkylated proteins were subjected to electrophoresis in the presence of SDS on a minigel consisting of 7% polyacrylamide. Proteins were transferred to polyvinylidene difluoride membranes (Matsudaira, 1987). The protein band which migrated with an *M*<sub>r</sub> of 110K was cut out for sequencing. An acetyl blocking group on the amino terminus of AmEPV spheroidin was removed using anhydrous trifluoroacetic acid (Wellner et al., 1990). The deblocked protein was sequenced by automated Edman degradation on an Applied Biosystems Model 470A gas phase sequencer (Hewick et al., 1981). Internal sequences were determined from peptides following lys-c endoprotease digestion. Prior to sequencing, peptide fragments were resolved on a Brownlee HPLC microbore column (RP-300, 2.1 mm × 30 mm) using an Applied Biosystems 130A Separation System.

**Identification of DNA clones corresponding to the AmEPV spheroidin gene.** Two degenerate oligonucleotide probes were constructed from the peptide sequence data. AA(C/T)GTICCNCTICNACIAA, corresponding to NVPLATK, and TA(C/T)ACIAAC(T/C)TT(C/T)ACNAA, corresponding to YTNTFK, where N is any one of the four nucleotides and C represents the non-hydrogen-bonding nucleotide base inosine. Plasmids containing cDNA inserts derived from mRNA were digested with EcoRI, and DNA was resolved on a 0.7% agarose gel by electrophoresis and transferred to nitrocellulose by Southern blotting. These blots were subsequently probed with the above oligonucleotides which had been labelled at their 5′ ends with T4 polynucleotide kinase and [γ-32P]ATP. Hybridization was overnight at 37 °C in buffer containing 6 × SSC and 100 µg/ml yeast tRNA and washing was performed with 2 × SSC at room temperature. Genomic libraries were subsequently probed with positive clones from the mRNA library. DNA sequencing of cDNA and cloned genomic fragments was performed on double-stranded plasmid DNA using synthetic oligonucleotide primers, [α-32P]dATP (Amersham, 3000 Ci/mmol) and the T7 DNA polymerase sequencing kit from Pharmacia. Both DNA strands were sequenced to ensure accuracy.

**Results**

**Cultivation of AmEPV in cultured insect cell lines and isolation of occlusion bodies**

At the start of our studies with AmEPV we had difficulty propagating the virus in BTI-EAA cells. Occlusion bodies appeared in only a proportion of the cells even after 1 week of incubation. Therefore, the cell line was cloned using a fluorescence-activated cell sorter to produce homogeneous populations which supported the replication of the entomopoxivirus and permitted the formation of occlusion bodies. Three selected cell lines totally supported the replication of the virus and a typical illustration of an AmEPV infection in culture is shown in Fig. 1(a). Eventually all the cells became round and each contained 20 to 30 cytoplasmic occlusion bodies. We also observed that reducing the level of FCS from 10% to 1% during infection dramatically accelerated the process of occlusion body formation. This finding may be related to the observation by other investigators that epidermal growth factor inhibits vaccinia poxvirus infection through competition for cellular receptors (Eppstein et al., 1985). A gypsy moth cell line (IPLB-LD-652) also efficiently supported infections (Goodwin et al., 1990). Both cell lines yielded similar titres of virus (10<sup>7</sup> to 10<sup>8</sup> p.f.u./ml) but the occlusion bodies in IPLB-LD-652 cells were one-quarter of the diameter of those produced in BTI-EAA cells. Growth of AmEPV in the gypsy moth cells was slightly faster (by 24 h), possibly due to the fact that virus was cultured in serum-free medium. Occlusion bodies of AmEPV (shown in Fig. 1(b) were isolated from infected BTI-EAA cells as described in Methods and used in the subsequent experiments.
Comparison of AmEPV and ChEPV occlusion body proteins by SDS–PAGE

Approximately 10⁶ occlusion bodies from AmEPV and CbEPV infections were solubilized in sample buffer containing 5% 2-mercaptoethanol and subjected to electrophoresis on SDS–polyacrylamide gels as shown in Fig. 2. The migration of protein bands for the two types of occlusion bodies was different. The major occlusion body protein of CbEPV consists of a 47K monomer which tends to aggregate in the absence of reducing agent (Yuen et al., 1990). The process of monomer formation was reversible and dimers could be reformed by removing 2-mercaptoethanol by dialysis. Peptide antibodies directed against the carboxy terminus of monomeric spheroidin also reacted with the larger aggregates (unpublished data) and the amino termini of both the monomer and dimers were shown to be identical by gas-phase sequencing techniques. In contrast, it was demonstrated that the occlusion bodies of AmEPV were composed primarily of a 114K polypeptide with minor protein bands being present at 38K and 16K, as shown in Fig. 2. To determine the primary sequence of AmEPV spheroidin and compare it to that of the CbEPV analogue, AmEPV spheroidin was gel-purified and sequenced by Edman degradation as described in Methods. Its amino terminus was found to be SNVPLA?KT1?K; six internal peptides [ELLFP?NV-NEAQP?KYV, ISEYTNFTKS, LVDSVSQSQ-DVLQGLLNT(E/C)NTID, ASRLGNGLVLVNRIN-(E/C)SN, GYRGVYENNN and IFD(E/C)NPNNN] were also sequenced following lys-c endoprotease digestion. None of these amino acid sequences was found in the protein sequence for the CbEPV occlusion body protein published previously (Yuen et al., 1990).

Antibodies directed against AmEPV and CbEPV occlusion body proteins do not cross-react

Antisera against all the solubilized occlusion body proteins from AmEPV and the gel-purified 47K occlusion body protein of CbEPV were produced in rabbits. A closely related entomopoxvirus (CfEPV) of another species of spruce budworm (C. fumiferana) was also being studied in our laboratories. The three different types of occlusion bodies were solubilized in the presence of reducing agent and the proteins were resolved on SDS–polyacrylamide gels. Immunoblots were probed with the anti-AmEPV and anti-CbEPV antibodies, and binding was detected with ¹²⁵I-labelled Protein A (data not shown). Anti-AmEPV antibodies reacted only with the AmEPV occlusion body proteins and failed to react with the 47K spheroidin protein of CbEPV and CfEPV. Antibodies directed against the 47K protein of CbEPV failed to react with AmEPV occlusion body proteins, and reacted primarily with both the major occlusion body proteins from CbEPV and CfEPV. Although the occlusion body proteins of CbEPV and CfEPV were similar antigenically, the spruce budworm entomopoxvirus occlusion body protein was clearly unrelated to the proteins of AmEPV.

Southern blot analysis of entomopoxvirus genomic DNA with probes constructed from major occlusion body genes of CbEPV and AmEPV

Genomic DNA was purified from occlusion bodies of the two different entomopoxviruses and digested with restriction endonucleases (EcoRI, HindIII or XmnI, or a combination of the three). DNA fragments were subsequently resolved on agarose gels. Radioactive probes were constructed from either the major occlusion
Entomopoxvirus occlusion body protein

Fig. 3. Nucleotide sequence of the AmEPV spheroidin gene and its predicted protein product. Peptides derived directly by N-terminal sequencing are underlined once. A putative leucine repeat (leucine zipper) region is underlined twice. The nucleotides of the gene and its immediate flanking sequences are numbered from 1 to 3360.
body gene of CbEPV or that of AmEPV, and hybridized individually to Southern blots containing both CbEPV and AmEPV genomic fragments. Blots were washed at low stringency and exposed to X-ray film (data not shown). Only DNA specific for CbEPV was detected with the probe derived from the major occlusion body gene of CbEPV. Conversely, the probe specific for the spheroidin gene of AmEPV failed to hybridize with genomic fragments from CbEPV. The preceding results were the first indication that the CbEPV major occlusion body gene and the AmEPV spheroidin gene differed at both the level of nucleic acid and encoded protein.

Cloning and predicted sequence of the AmEPV spheroidin protein

Degenerate oligonucleotides were constructed from the partial protein sequence data derived by N-terminal sequencing as outlined in Methods. A cDNA library was synthesized from the mRNA of IPLB-LD-652 cells infected with AmEPV and probed with these 32P-labelled oligonucleotides. Several clones which contained inserts of 1900 to 2200 bases were identified and sequenced. The nucleotide sequence of one clone (Am93) was deduced to contain an open reading frame (ORF) which encoded the amino-terminal peptide forecast by N-terminal sequencing. Both strands of this clone were sequenced in their entirety and the predicted polypeptide contained two other peptide sequences corresponding to N-terminal sequences determined previously. This clone contained a unique internal XmnI site in the open reading frame. A genomic library consisting of XmnI fragments of AmEPV DNA cloned into pUC19 was probed with EcoRI-XmnI fragments from the cDNA clone Am93. Genomic clones containing the 5' and 3' portions of the AmEPV spheroidin gene were identified and sequenced. An ORF of 3009 nucleotides was determined to encode spheroidin. The 5' flanking (1183 nucleotides) and 3' flanking (470 nucleotides) regions of the gene were also determined (data not shown). The sequences of the flanking and coding regions have been submitted to GenBank. The DNA sequence of the AmEPV spheroidin gene and its predicted polypeptide are also shown in Fig. 3.

The predicted product of the AmEPV gene consists of 1003 amino acid residues which specify an unmodified protein of Mr 114881 with an isoelectric point of 6.3. AmEPV spheroidin contains 38 cysteine residues and 14 potential Asn-linked glycosylation sites. An interesting feature of the protein is a leucine zipper pattern located between amino acids 119 and 140, which could be important in forming protein aggregates. This leucine repeat pattern is indicated by the double underlining in Fig. 3. Six basic amino acid regions were located between residues 9 and 13, 17 and 26, 320 and 345, 396 and 418, 675 and 697, and 927 and 946, with acidic regions between residues 380 and 394 and 470 and 478. These regions may be important for charge interactions during aggregation of the monomers. A potential hydrophobic membrane-spanning region exists between amino acids 631 and 647 but its significance is unclear. Overall, AmEPV spheroidin had the properties of a globular protein with a propensity to form short domains of a-helix and with scattered hydrophobic regions over the first 800 amino acids. The final 200 amino acids at the carboxy terminus are entirely hydrophilic. This spheroidin molecule showed no similarity to the analogous protein of CbEPV, the A-type inclusion (ATI) body protein of cowpox virus or the polyhedrin proteins of baculovirus. However, the major occlusion body proteins of poxviruses all have a large number of cysteine residues: 38 for AmEPV, nine for CbEPV and 26 for the ATI body protein of cowpox virus. The cysteines of AmEPV spheroidin were clustered mainly at the beginning, in the middle and at the very end of the protein. A search against sequences in the GenBank database revealed that the AmEPV sequence was unique.

The late gene promoters of poxviruses are characteristically rich in A and T residues and contain a TAAAT element which overlaps the AUG initiation codon for the majority of late proteins (Davison & Moss, 1989). This element specifies 5' polyadenylation of poxvirus mRNA. These late promoters usually extend about 27 nucleotides upstream from the TAAAT sequence. The 5' upstream region of the AmEPV spheroidin gene is a classic example of a poxvirus late promoter and bears a resemblance to many of the late gene promoters of vaccinia virus (Davison & Moss, 1989). It is A+T-rich and possesses a TAAAT element adjacent to its initiation codon. This promoter region extends 31 bases upstream before a C residue is encountered. Our laboratory has demonstrated previously that the CbEPV spheroidin promoter consists of 33 bases which could function as a strong late promoter in the vaccinia virus system (Pearson et al., 1991).

Discussion

This publication reports the sequence of the gene for the major occlusion body protein (spheroidin) of AmEPV. We found the predicted protein to differ substantially from the major occlusion body protein of CbEPV, the sequence of which has been determined previously (Yuen et al., 1990). The CbEPV protein has an Mr, of 47K and an isoelectric point of 5.01, but also possesses a large number of evenly spaced cysteine residues. Some
mammalian viruses, such as cowpox virus, are also capable of forming occlusion bodies in their respective host cells (Patel et al., 1986). These occlusion bodies are irregular in shape and are called ATI bodies. These ATIs are composed of a single protein of 160K, the gene encoding which has been cloned and sequenced recently (Funahashi et al., 1988). The predicted cowpox virus occlusion body protein contains 26 cysteine residues and 11 potential sites for N-glycosylation, exhibits an abundance of basic and acidic amino acids, and has an isoelectric point of 4.87. With the exception of a large number of cysteine sulphhydril groups, no homology was apparent when the ATI protein was compared to AmEPV or CbEPV occlusion body proteins. However, the ATI protein gene promoter is a typical late poxvirus gene promoter and contains the TAAATG consensus sequence found in the AmEPV spheroidin gene promoter. The A+T-rich region of the ATI protein gene promoter is shorter; 23 nucleotides compared to 31 nucleotides for the AmEPV promoter. The extensive GAT repeats of the cowpox virus promoter are also not present in the region upstream of the AmEPV spheroidin promoter.

Vaccinia virus contains an analogous protein (94K) which is a truncated version of the ATI protein (Patel et al., 1986). The 94K protein accumulates in large quantities late in infection, but is not capable of aggregating to form inclusion bodies. The deletions which have produced this smaller protein may be a result of evolutionary divergence. From these findings, we conclude that the major occlusion body proteins of three poxviruses are different. Their only similarity seems to be a high content of cysteine, and the physical ability of the protein to aggregate and include virions. Other investigators have demonstrated that the occlusion proteins of some insect cytoplasmic polyhedrosis viruses also differ substantially from one another (Fossiez et al., 1989). A previous publication from our laboratory (Yuen et al., 1991) has indicated that another CbEPV gene product, nucleoside triphosphate phosphohydrolase 1, shares significant similarity with the analogous protein of vaccinia virus. Its gene promoter also exhibits the motifs characteristic of the late gene promoters for poxviruses. Despite the lack of homology between occlusion body proteins, these findings still support the classification of entomopoxviruses within the family of poxviruses.

We initiated our work on entomopoxviruses with the ultimate intent of harnessing the strong promoters of the spheroidin gene for purposes of constructing a new family of insect virus expression vectors analogous to the baculovirus system (Luckow & Summers, 1988). This concept is presently being tested in our laboratory. In addition, the promoter from the spheroidin gene of CbEPV has already been inserted into a vaccinia virus expression vector and determined to function fivefold better than the 7.5K protein promoter normally used in many vaccinia virus vectors (Pearson et al., 1991). A similar approach was suggested and adopted using the cowpox virus ATI protein gene promoter described above to direct higher levels of expression from vaccinia virus recombinants in mammalian cells (Funahashi et al., 1988; Patel et al., 1988). Very little is known concerning the molecular biology of entomopoxviruses, and the viral proteins of AmEPV and CbEPV have not been clearly defined. The existence of permissive cell lines is enabling these studies to be pursued.

References


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