Dissimilar expression of Autographa californica multiple nucleocapsid nuclear polyhedrosis virus polyhedrin and p10 genes


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The temporal expression of the Autographa californica multiple nucleocapsid nuclear polyhedrosis virus polyhedrin and p10 genes in Spodoptera frugiperda cells was studied using virus recombinants in which either gene was replaced by the juvenile hormone esterase (JHE) gene of Heliothis virescens. The JHE served as a highly specific and sensitive reporter for gene expression. Activation of the p10 gene followed a pattern different to that of polyhedrin. The p10 gene was activated a few hours earlier than the polyhedrin gene, but its expression reached a lower maximum level. Northern blot analysis complemented and confirmed the results obtained from the JHE assays. Co-infection of sense recombinants and those containing an antisense copy of the JHE gene in place of the polyhedrin or p10 gene resulted in reduced levels of JHE gene expression. These experiments independently supported the hypothesis that the p10 gene promoter is more active at earlier times post-infection than that of the polyhedrin gene. The results also highlight the potential of the antisense strategy as an experimental approach for the study of baculovirus gene regulation and possibly insect metabolism.

Introduction

The baculoviruses are a large group of insect DNA viruses with covalently closed dsDNA genomes. They are pathogenic for insects and are being used as biological insecticides (Granados & Federici, 1986). Baculoviruses, notably the multiple nucleocapsid nuclear polyhedrosis viruses (MNPV) of Autographa californica (Ac) and Bombyx mori, are also exploited as vectors for the high level expression of foreign genes (Luckow & Summers, 1988; Miller, 1988; Maeda, 1989). The sequential and ordered transcription and expression of different sets of baculovirus genes (for a review see Friesen & Miller, 1986) suggest a replication cycle that consists of four, more or less temporally distinct phases (α, β, γ and δ; Kelly & Lescott, 1981).

The virus replication cycle results in the production of two virus forms. Single nucleocapsid-containing virus particles that bud from infected cells are produced in the third phase. These extracellular viruses are infectious for insect cells in vitro and in vivo. They disappear when polyhedra with the multiple nucleocapsid-containing virions begin to appear in the nuclei (Volkmann & Keddie, 1990). The synthesis of polyhedra, occlusion bodies that mediate infection of insects in nature, marks the fourth and very late phase of the replication cycle (for a review see Blissard & Rohrmann, 1990). In addition to the gene for polyhedrin, other genes involved in polyhedron morphogenesis and cell lysis are expressed during this last phase.

During the very late phase of the infection two viral gene products, polyhedrin and p10, are expressed in copious quantities (Smith et al., 1982). Polyhedrin is the major constituent of polyhedra; p10 is a major component of so-called fibrillar structures in infected cells (van der Wilk et al., 1987) and is thought to play a role in cell lysis, supporting the release of polyhedra from cells and tissues (Williams et al., 1989). Baculovirus expression vector systems are based on the fact that the polyhedrin and p10 genes are dispensable for virus replication (Fraser et al., 1985; Vlak et al., 1988) and can be replaced by foreign genes.

The time of onset and temporal regulation of polyhedrin and p10 gene expression are a matter of debate (Friesen & Miller, 1986). It is generally assumed that the very late phase commences around 18 h post-infection (p.i.) (Kelly & Lescott, 1981). However, in AcMNPV-infected cells the polyhedrin protein has been detected as early as 8 h p.i. by radioimmunoassays (Maruniak & Summers, 1981), whereas p10 is found around 10 to 12 h...
p.i. using PAGE (Williams et al., 1989). Recent experiments using AcMNPV–β-galactosidase recombinants have indicated that polyhedrin and p10 promoter-driven expression starts between 12 and 18 h p.i. (Weyer et al., 1990), and Northern blot analysis has suggested that p10 mRNA is synthesized later in infection (10 to 12 h p.i.) (Rohel et al., 1983) than polyhedrin mRNA (6 to 8 h p.i.) (Friesen & Miller, 1986). However, these studies suggested distinct transcription kinetics for polyhedrin and p10 mRNA.

In this report we investigated the temporal expression of the AcMNPV polyhedrin and p10 genes in Spodoptera frugiperda cells in more detail by using two novel approaches. First, we exploited a sensitive marker for gene expression, the Heliothis virescens juvenile hormone esterase (JHE; Hammock et al., 1990). When expressed via a baculovirus vector, this enzyme is secreted by insect cells and hence is easy to assay in the medium of infected cells. We have constructed AcMNPV recombinants expressing the JHE gene under the control of either the polyhedrin or p10 promoter, which enabled us to evaluate the timing of transcription and expression of these very late genes in insect cells, and to correlate these two processes. Second, we employed an antisense strategy to determine which of the promoters is activated first. To this end we constructed AcMNPV recombinants with the JHE gene in the antisense orientation downstream from the polyhedrin or p10 promoter, and carried out dual infections with recombinants carrying the JHE gene in the sense and antisense orientations.

Methods

VIRUSES AND INSECT CELLS. The S. frugiperda cell line IPLB-SF-21 (Vaughn et al., 1977) was used and maintained in plastic tissue culture flasks in TMN-FH medium (Hink, 1970) supplemented with 10% foetal calf serum. For protein expression studies this cell line was adapted to Ex-Cell 400 medium (JR Scientific).

AcMNPV strain E2 (Smith & Summers, 1978) was used as the wild-type (wt) virus. Recombinants AcMNPV/DZ1 and AcMNPV/AS3 served as control viruses lacking the polyhedrin and p10 gene coding sequences, respectively (Zuidema et al., 1990; Vlak et al., 1990). All recombinants contained a lacZ gene cassette to facilitate screening. The infectivity of non-occluded virus (NOV) was determined using an endpoint dilution method (Vlak, 1979). The titres were calculated as TCID₅₀/ml medium. The infectivity endpoint of recombinants was determined using X-Gal (25 μg/ml) as a β-galactosidase activity indicator.

ANTIBODIES. A rabbit polyclonal antiserum was raised against purified H. virescens JHE by affinity chromatography (Abdel-Aal & Hammock, 1985).

Plasmids and transfer vectors. The AcMNPV transfer vectors pAcDZ1 (Zuidema et al., 1990) and pAcAS3 (Vlak et al., 1990) lack the polyhedrin gene and p10 gene coding sequences, respectively. These vectors contain flanking sequences to facilitate recombination, a lacZ gene cassette and a unique BamHI cloning site downstream of the wt polyhedrin or p10 promoter for insertion and transcriptional fusion of foreign genes. The lacZ gene cassette consists of the Drosophila melanogaster heat shock promoter hsp70, the bacterial lacZ gene and the simian virus 40 (SV40) dual transcription termination sequence. The BamHI site was used to insert the JHE gene in the sense and the antisense orientations into the polyhedrin or p10 locus. Plasmid pJHE16B contains a 1.7 kb BglII insert encoding the JHE gene of H. virescens (Hammock et al., 1990).

Construction of AcMNPV transfer vectors containing the JHE gene. Plasmid pJHE16B was digested with BglII, and a 1.7 kb fragment containing the sequence encoding the H. virescens JHE gene was isolated from a 0.7% agarose gel. This fragment was ligated into the BamHI site of transfer vectors pAcDZ1 and pAcAS3 to give vectors with the JHE gene in the sense (pAcPR3 and pAcPR1) or antisense (pAcPR4 and pAcPR2) orientation under control of the polyhedrin (pAcPR3 and pAcPR4) or p10 (pAcPR1 and pAcPR2) promoter (Fig. 1). Digestion, ligation and transformation procedures were as described by Sambrook et al. (1989). Plasmid DNA was recovered from transformed Escherichia coli cells by the alkaline lysis method of Birnboim & Doly (1979) followed by CsCl centrifugation.

INFECTION AND TRANSFECTION OF INSECT CELLS. S. frugiperda cells were cotransfected with wt AcMNPV DNA and transfer plasmids using the calcium phosphate precipitation technique essentially as described by Smith et al. (1983) with some minor modifications (Vlak et al., 1988). Recombinant plaques were identified by development of a blue colour upon addition of X-Gal (diluted 1:5 in culture medium; X-Gal stock, 25 μg/ml). Putative recombinants were plaque-purified four times to genetic homogeneity (Vlak et al., 1990). Cells were infected with the NOV form of the virus at a multiplicity of 20 TCID₅₀/cell (Zuidema et al., 1990).

DNA, RNA AND PROTEIN ANALYSIS. DNA obtained from plasmids or from extracellular NOVs of wt and recombinant AcMNPV was subjected to restriction enzyme analysis and Southern blot hybridization as described by Sambrook et al. (1989). RNA isolation from cell cultures, at 6 h intervals until 60 h p.i. (time zero corresponds to the time of addition of the inoculum to the insect cells), and Northern blot analysis were carried out according to Sambrook et al. (1989). Glyoxylated RNA was resolved in 1% agarose gels in 10 mM-sodium phosphate buffer, transferred to a GeneScreen Plus membrane and hybridized to a random-primed 32P-labelled probe derived from an internal 120 bp-long Smal–PstI fragment of the JHE gene (Hanzlik et al., 1989).

For protein analysis the supernatant of uninfected and infected S. frugiperda cells cultured in Ex-Cell 400 medium was collected. Since the NOVs present in the supernatant complicated the overall protein picture and are unnecessary for Western blot analysis, they were removed by centrifugation for 1 h at 25000 r.p.m. in an SW41 rotor. The supernatant was analysed by SDS–PAGE in a 12–5% gel using the method of Laemmli (1970) in a Bio-Rad PROTEAN II apparatus. The gels were stained with Coomassie blue or subjected to Western blot analysis.

Western blot analysis was performed as described by Zuidema et al. (1989) with some modifications. After overnight treatment of the blot with milk powder (0.2% v/v) at 4 °C to prevent non-specific antibody binding, the blot was incubated for 1 h at room temperature with a polyclonal anti-JHE antiserum (Hanzlik & Hammock, 1987). Since this antiserum had been shown to cross-react with an Ex-Cell 400 medium protein in the size range of the JHE protein, it was preincubated with 0.2% (v/v) milk powder in this medium to complex the antibodies against cross-reacting protein. This eliminated cross-reactivity on the blot.

ACTIVITY OF JHE. Samples (100 μl) were taken from the medium of infected cells (approximately 10⁷ cells infected with 10 TCID₅₀/cell) to
determine the JHE activity using a partition assay (Hammock & Roe, 1985). Activity was monitored by incubation for 15 min at 30 °C with a mixture of tritiated racemic juvenile hormone III (JHIII) (NEN; 11.6 Ci/mmol) and unlabelled racemic JHIH (Calbiochem) at a final substrate concentration of 5 × 10⁻⁶ M, under conditions in which the extent of hydrolysis was proportional to time and protein concentration, and the enzyme activity approached V_max conditions. To meet these conditions the samples were, depending on the time p.i. at which they were taken, diluted 50-fold (times 0, 6, 9, 10, 11 and 13 h p.i.), 100-fold (times 15 and 18 h p.i.), 400-fold (times 22 and 26 h p.i.), 500-fold (times 48 h p.i.) or 750-fold (times 60, 72 and 86 h p.i.) in 0.2 M-sodium phosphate buffer pH 7.4. From these dilutions, 100 μl samples were assayed in triplicate. Each expression experiment was repeated twice. Therefore the data in Table 1 and Fig. 2 are the mean of nine experiments. The enzyme activity was determined using the partition assay and applying the JHE activity formula derived by Hammock & Roe (1985). Activity was monitored by incubation for 15 min at 30 °C with 0.2 M-sodium phosphate buffer pH 7.4. From these dilutions, 100 μl samples were assayed in triplicate. Each expression experiment was repeated twice. Therefore the data in Table 1 and Fig. 2 are the mean of nine experiments. The enzyme activity was determined using the partition assay and applying the JHE activity formula derived by Hammock & Roe (1985). A JHE activity of 100 nmol/min/ml corresponds to about 75 mg JHE/litre of medium (Hammock et al., 1990).

Results

Construction of AcMNPV recombinants containing the JHE gene

H. virescens JHE has been shown to be a sensitive and reliable marker for protein expression by baculovirus recombinants (Hammock et al., 1990). We exploited the properties of this enzyme to compare the temporal expression of polyhedrin and p10 in infected S. frugiperda Sf21 cells. The JHE gene (Hanzlik et al., 1989) was cloned in the sense (Fig. 1) and antisense (not shown) orientations into the transfer vectors pAcDZ1 and pAcAS3. These vectors were designed for convenient screening of recombinants and the high-level expression of foreign genes under the control of the polyhedrin or p10 promoter. The orientation of the JHE inserts in the subsequent series (pAcPR) of transfer vectors was confirmed by restriction endonuclease analysis and by nucleotide sequencing of the junction between the polyhedrin or p10 promoter and the JHE gene. S. frugiperda cells were transfected with the pAcPR transfer vectors together with wt AcMNPV DNA, and recombinants were selected and further plaque-purified on the basis of β-galactosidase expression. Restriction endonuclease digestion of infected cell DNA confirmed the correct insertion of the JHE gene in the DNA of the various recombinants (data not shown).

Expression analysis of JHE in AcMNPV/PR3- and AcMNPV/PR1-infected S.frugiperda cells

The JHE protein has a signal sequence at the N terminus (Hanzlik et al., 1989) that directs the secretion of at least 95% of the JHE produced into the cell culture medium (Hammock et al., 1990). This facilitates the study of the temporal expression of the JHE gene under the control of the p10 and polyhedrin promoters. S. frugiperda cells were infected with AcMNPV/PR1 or AcMNPV/PR3 at the same multiplicity, and expression was measured as JHE activity in the culture medium. JHE activity above background was first detected with AcMNPV/PR1-infected cells at 11 h p.i., whereas AcMNPV/PR3-infected cells started to produce JHE at approximately 15 h p.i. (Table 1, Fig. 2). This suggested that the p10 gene is active at least 4 h earlier than the polyhedrin gene.

<table>
<thead>
<tr>
<th>Time (h p.i.)</th>
<th>AcMNPV/PR1</th>
<th>AcMNPV/PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02 ± 0.02</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.02 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>11</td>
<td>0.10 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>13</td>
<td>0.44 ± 0.06</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>0.75 ± 0.10</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>18</td>
<td>2.25 ± 0.30</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>22</td>
<td>4.50 ± 0.25</td>
<td>1.25 ± 0.13</td>
</tr>
<tr>
<td>26</td>
<td>12.10 ± 1.50</td>
<td>4.68 ± 1.00</td>
</tr>
</tbody>
</table>

* All results are expressed as nmol JHE degraded/min/ml ± S.D. and are the average of three assays on 100 μl samples of supernatant. Each experiment was repeated twice. The standard deviation given for time zero is based on four assays of two samples.
† Figures in bold represent levels of expression significantly above background levels.

Expression of AcMNPV polyhedrin and p10 genes

Table 1. JHE gene expression in cells infected with AcMNPV/PR1 and AcMNPV/PR3*
Fig. 1. Schematic diagram of the construction of transfer vectors pAcPR1 and pAcPR3 containing the JHE gene in the sense orientation (see Methods). B, BamHI; S, SmaI; H, HindIII; Xb, XbaI; E, EcoRI; X, XhoI; Sp, SphI; C, ClaI; Bg, BglII; php, polyhedrin promoter; term, termination signal; p10p, p10 promoter; Ac, AcMNPV DNA.

PAGE and Western blotting a single major band of about 69K was detected in medium from AcMNPV/PR1- and AcMNPV/PR3-infected cells (Fig. 3). This was the size expected on the basis of the DNA sequence data (62K) (Hanzlik et al., 1989), taking into account four potential glycosylation sites in JHE. The minor lower Mr bands most likely represented breakdown products. The amounts of JHE, as deduced from the intensity of the 69K protein bands, were approximately the same, although the amount in AcMNPV/PR1-infected cells was slightly less compared to that in AcMNPV/PR3-infected cells. This confirmed the deductions made from the JHE activity measurements (Fig. 2), i.e. that the polyhedrin promoter-containing recombinant produced more JHE than the p10 promoter-containing recombinant.
Expression of AcMNPV polyhedrin and p10 genes

Fig. 2. Time course of JHE production in S. frugiperda cells infected with AcMNPV/PR3 (---) and AcMNPV/PR1 (—). Each point represents the median of three separate determinations. Each experiment was repeated twice. Standard deviation is indicated.

Fig. 3. Western blot analysis of the supernatant of S. frugiperda cells infected with wt AcMNPV, AcMNPV/AS3, AcMNPV/PR1, AcMNPV/DZ1 and AcMNPV/PR3 (lanes 1 to 5) at a multiplicity of 20 TCID50/cell. The position of JHE is indicated (69K). The sample in each lane is equivalent to the supernatant of 4 x 10^3 cells.

Northern blot analysis of RNA from AcMNPV/PR3- and AcMNPV/PR1-infected S. frugiperda cells

To investigate whether the temporal synthesis of JHE by polyhedrin and p10 promoter-containing recombinants is a reflection of transcription, Northern blot analysis was performed on total RNA isolated from S. frugiperda cells infected with AcMNPV/PR1 and AcMNPV/PR3 (Fig. 4) at various times after infection. JHE gene-containing transcripts were observed as early as 12 h p.i. in AcMNPV/PR1-infected cells, whereas polyhedrin promoter-driven expression of JHE was detected from 15 h p.i. This pattern correlated well with significant JHE activity in the culture medium (Table 1). The Northern blot data supported the hypothesis that the p10 gene becomes more active at least a few hours before the polyhedrin gene. After 48 h p.i. the amount of p10 promoter-driven JHE transcript levelled off or decreased (Fig. 4), whereas the amount of polyhedrin promoter-driven JHE transcript continued to increase. These observations were confirmed by RNA dot-blot hybridization using the same probe as in the Northern blot analysis (data not shown).

Upon over-exposure of the autoradiogram, both p10 and polyhedrin promoter-derived JHE mRNA became visible 6 h p.i. (data not shown). The length of the mRNA observed at 6 h p.i. corresponded to the length of the mRNA observed at later times. However, this onset of transcription did not correlate with significant JHE activity in the culture medium (see Table 1).

In addition to the major JHE transcript of approximately 1-8 kb, a much larger transcript was also observed in recombinant-infected cells (Fig. 4 and 5). In AcMNPV/PR3-infected cells this transcript was about 6-0 kb, whereas in AcMNPV/PR1-infected cells a transcript of 5-7 kb was found. These transcripts also hybridized to lacZ-specific probes (data not shown) and most likely represented transcripts that failed to terminate at the SV40 termination sequence, read, in an antisense fashion, through the lacZ gene and the hsp70 fragment, and terminated at the authentic polyhedrin and p10 gene transcription termination signals.

Further analysis of polyhedrin and p10 promoter-driven transcription

To analyse the onset of transcription from the polyhedrin and p10 promoters in greater detail, AcMNPV recombinants were constructed which had the JHE gene in the
Fig. 5. Northern blot analysis of total RNA isolated 48 h p.i. from S. frugiperda cells infected with AcMNPV/PR1 (lane 1), AcMNPV/PR2 (lane 2), AcMNPV/PR3 (lane 3) and AcMNPV/PR4 (lane 4). The RNA was hybridized as in Fig. 4. The sizes of the transcripts are indicated. Each lane contains 4 μg glyoxylated RNA.

antisense orientation behind the polyhedrin (AcMNPV/PR4) or p10 (AcMNPV/PR2) promoter. Northern blotting of total cell RNA indicated that JHE gene transcripts were present in cells infected with sense (Fig. 5, lanes 1 and 3) and antisense (Fig. 5, lanes 2 and 4) recombinants in similar amounts, and were of the same approximate length, about 1-8 kb. This size was expected because the 1-7 kb JHE gene insert does not contain canonical transcription termination signals in both strands of the nucleotide sequence (Proudfoot & Brownlee, 1976; Hanzlik et al., 1989) and therefore should terminate in the SV40 termination sequence. When probed with an antisense-specific JHE gene probe, only the samples in lanes 2 and 4 of Fig. 5 showed a transcript signal (data not shown).

The antisense JHE RNA-producing viruses AcMNPV/PR2 (p10) and AcMNPV/PR4 (polyhedrin) were used in experiments designed to substantiate our observation that p10 gene transcription initially is maintained at a higher level than polyhedrin gene transcription. The rationale behind these experiments was that earlier activation of the p10 gene would produce antisense transcripts that would interfere with polyhedrin promoter-directed JHE gene expression. However, polyhedrin promoter-driven JHE gene antisense transcripts would have a less pronounced effect on p10 promoter-driven JHE gene transcripts.

To test this hypothesis, cells were co-infected with AcMNPV recombinants with the JHE gene transcribed from the polyhedrin and p10 promoter in the sense and antisense orientation (at a ratio of 1:4), and harvested at 1, 16, 20 and 24 h p.i. The m.o.i. of each type of recombinant was kept constant at 20. Cultures infected with AcMNPV/PR1 and AcMNPV/AS3 (p10−; Vlak et al., 1990) or AcMNPV/PR3 and AcMNPV/DZ1 (polyhedrin−; Zuidema et al., 1990) at the given ratio served as 100% controls for JHE gene activity in the absence of antisense RNA. At early times p.i. a mixed infection of AcMNPV/PR1 and AcMNPV/PR4 showed virtually no delay in the onset of JHE gene expression, whereas in a mixed infection of AcMNPV/PR3 and AcMNPV/PR2, JHE gene expression was delayed by several hours (data not shown). This suggested that the antisense RNA is effective in blocking either transcription or translation.

Therefore we tested this effect at constant m.o.i. and sense : antisense ratios ranging from 1:1 to 1:19 (Table 2). At 48 h p.i. samples of 100 μl were taken from the supernatant and the JHE activity was determined as described in Methods. In the homologous combination of AcMNPV/PR1 and AcMNPV/PR2 there was a marked effect of the antisense virus at all ratios tested. Co-infection of cells with AcMNPV/PR1 and the antisense polyhedrin virus AcMNPV/PR4 showed that inhibition of JHE gene expression was far less pronounced. A reverse effect was apparent in co-infection of AcMNPV/PR3 with the homologous antisense virus or AcMNPV/PR2. The latter p10 promoter-containing antisense virus inhibited JHE gene expression more strongly than AcMNPV/PR4. These results indicated

Table 2. Effect of antisense transcripts on the expression of the JHE gene in infected S. frugiperda cells

<table>
<thead>
<tr>
<th>Sense : antisense ratio</th>
<th>PR1†</th>
<th>PR1</th>
<th>PR3</th>
<th>PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1:1</td>
<td>77‡</td>
<td>97</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>1:4</td>
<td>60</td>
<td>71</td>
<td>69</td>
<td>40</td>
</tr>
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<td>1:9</td>
<td>35</td>
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<tr>
<td>1:19</td>
<td>29</td>
<td>69</td>
<td>53</td>
<td>37</td>
</tr>
</tbody>
</table>

* AcMNPV sense recombinants expressing the JHE gene under the control of the polyhedrin or p10 promoter were diluted at various ratios with JHE antisense recombinants. The final m.o.i. was maintained at 20 TCID<sub>50</sub>/cell. The level of JHE gene expression was compared to that in a similar experiment in which the antisense recombinants were replaced by their parental polyhedrin− (AcMNPV/DZ1; Zuidema et al., 1990) or p10− (AcMNPV/AS3; Vlak et al., 1990) recombinants (100% controls). Samples of 100 μl were taken from the supernatant and the JHE activity was determined as described in Methods. In the homologous combination of AcMNPV/PR1 and AcMNPV/PR2 there was a marked effect of the antisense virus at all ratios tested. Co-infection of cells with AcMNPV/PR1 and the antisense polyhedrin virus AcMNPV/PR4 showed that inhibition of JHE gene expression was far less pronounced. A reverse effect was apparent in co-infection of AcMNPV/PR3 with the homologous antisense virus or AcMNPV/PR2. The latter p10 promoter-containing antisense virus inhibited JHE gene expression more strongly than AcMNPV/PR4. These results indicated
that polyhedrin promoter-driven expression can be more efficiently blocked by p10 promoter-driven antisense transcripts than vice versa. This effect is more pronounced when the relative concentration of antisense recombinant is increased.

Discussion

The temporal expression of AcMNPV polyhedrin and p10 in S. frugiperda cells was studied using recombinants in which these two genes were replaced by the H. virescens JHE gene. This strategy eliminated the difficulty of comparing the levels of two proteins differing in size, amino acid sequence, stability and compartmentalization. In addition, this method reduced the possible influence of mRNA stability because polyhedrin and p10 promoter-driven JHE gene transcripts have an almost identical structure. Finally, JHE appears to be almost completely secreted (>95%) and is convenient to assay (Hammock et al., 1990).

The construction of recombinants with either the polyhedrin or p10 gene replaced by the JHE gene was chosen to allow direct comparison of JHE produced by recombinant viruses using different promoters. However, dual infections required accurate control of m.o.i., i.e. adequate determination of the infectivity of recombinant virus preparations. The novel screening system for polyhedrin (Zuidema et al., 1990) and p10 (Vlak et al., 1990) recombinants facilitated these determinations.

The regulation of baculovirus very late gene expression is not well understood. The promoters of the AcMNPV polyhedrin and p10 genes have a similar organization, but share only limited nucleotide sequence homology. The polyhedrin promoter consists of a 49 nucleotide 5' non-coding leader sequence before the translation initiation codon (Matsura et al., 1987; Rankin et al., 1988). A short sequence of about 20 nucleotides upstream from the transcription start site is probably involved in enhancement of transcription (Possee & Howard, 1987). Linker-scan mutation analysis of the polyhedrin promoter has failed to confirm this observation, but has identified sequences downstream of the canonical promoter element that may be crucial to initiation at the transcription start site (Ooi et al., 1989). The AcMNPV p10 promoter consists of a 70 nucleotide 5' non-coding leader sequence (Weyer & Possee, 1988; Qin et al., 1989) and an additional 30 nucleotides upstream from the transcriptional start with an enhancement function (Weyer & Possee, 1989). The 5' leaders of both promoters are extremely AT-rich (80%). A consensus nucleotide sequence (-AATAAGTATT-) is present, and includes a TAAG motif present in all baculovirus late and very late promoters (Rohrmann, 1986). Alterations in this motif abolish transcription (Rankin et al., 1988; Ooi et al., 1989; R. D. Possee, unpublished data).

Our experiments show that the p10 gene has a transcriptional pattern distinct from that of the polyhedrin gene, as suggested by Ooi et al. (1989), although it remains to be determined which promoter sequences are responsible for the differences in transcriptional activity of the polyhedrin and p10 genes. We hypothesize that the detection of JHE mRNA 6 h.p.i. reflects basal host cell RNA polymerase activity giving rise to non-translatable mRNA, possibly owing to the absence of some modifying group, e.g. a 5' cap. Our data also suggest that the polyhedrin gene is expressed at a higher level than the p10 gene. A recent expression study that sought to correlate polyhedrin and foreign gene expression with p10 transcription confirms our observation (Min & Bishop, 1991).

The experiments using antisense recombinants support the hypothesis that the increase in p10 gene activity occurs earlier than that in polyhedrin gene activity (Table 2), in accordance with Northern blot experiments (Fig. 4). Recombinants containing the p10 promoter also produce JHE earlier than those containing the polyhedrin promoter (Fig. 2). Late after infection, insect cells might become compromised and unable to process large amounts of glycosylated recombinant proteins (Jarvis & Summers, 1989). In view of this it may be more advantageous to engineer p10 promoter-containing recombinants for the expression of glycosylated proteins, as they will then be produced earlier in infection. Recombinants containing the p10 promoter may also be advantageous for the maintenance of the biological activity of recombinant proteins as well as their solubility (Murphy et al., 1990).

The mechanism of antisense RNA action is largely unknown. In prokaryotes some naturally transcribed RNA species regulate DNA replication or gene expression by hybridizing to their complementary target sequences (Mizuno et al., 1984). In bacteria antisense RNA inhibits translation by annealing to the mRNA and blocking ribosome binding. In higher organisms, the RNA duplex could potentially interfere with the normal processing of mRNA (splicing), its transport from the nucleus (Kim & Wold, 1985) or its translation in the cytoplasm (Crowley et al., 1985). Antisense RNA also occurs naturally in baculovirus-infected cells, in which polyhedrin mRNA appears to down-regulate a minor late RNA of unknown function (Ooi & Miller, 1990).

Experiments presented in this paper also provide a basis on which recombinants may be engineered, thus using an antisense strategy for the control of insects. Antisense RNA (cRNA or mRNA-interfering cRNA) has been employed in a number of systems (Green et al.,
1986) and proposed as a possible genetic approach for the prevention and treatment of disease in animals (Chang & Stoltzfus, 1985; Izant & Weintraub, 1984; Kim & Wold, 1985) and plants (Hemenway et al., 1988; Cuozzo et al., 1988; Powell et al., 1989). The antisense strategy may be exploited to block essential functions in the development of insects and hence serve as an alternative control strategy for insect pests. Baculoviruses could serve as vectors to target antisense RNA into the host organism.

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References


Expression of AcMNPV polyhedrin and pl0 genes


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