Baculovirus infection of Spodoptera exigua larvae: lacZ expression driven by promoters of early genes pe38 and me53 in larval tissue

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To follow the progression of infection of Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) within tissues of its larval host, we have constructed AcMNPV recombinants carrying lacZ reporter genes under the control of the early virus promoters pe38 and me53, in addition to the authentic genes. The early promoter–lacZ gene cassettes were located upstream of the very late polyhedrin gene. In infected insect cell lines, pe38 transcription is initiated at an early promoter, while me53 transcripts start from both early and late sites. Transcriptional mapping of the duplicated me53 and pe38 promoters driving lacZ expression showed that they initiated at the same start sites as in the authentic genes. Expression of lacZ by these recombinants was compared to a recombinant driving β-glucuronidase expression from the very late p10 promoter and lacZ expression from the constitutive heat shock protein 70 promoter of Drosophila melanogaster. After infection of Spodoptera exigua larvae with the different recombinants, we followed reporter gene expression and polyhedron formation in different tissues using immunohistochemistry and electron microscopy. LacZ expression, indicative of early viral transcriptional activity, was detected in nearly all larval tissues during the course of infection. In most tissues these early events were followed by pathophysiological changes associated with late and very late gene expression. However, p10 transcription and polyhedron formation were not observed in midgut goblet cells, Malpighian tubules and salivary glands. These results suggest that expression of early virus genes, such as me53 and pe38, is not restricted to larval tissues that are permissive for AcMNPV replication.

Introduction

The baculovirus Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) infects susceptible insects in their larval stage. The virus enters its lepidopteran hosts through midgut epithelial cells from where infection spreads throughout the insect. One characteristic of this virus is the production of two different structural forms that have distinct roles during infection of the host organism. Budded viruses serve to spread the infection within the larva, while polyhedra with occluded infectious nucleocapsids are required for survival in the environment. Infection of the larval midgut is the prerequisite for successful baculovirus infection (Keddie et al., 1989; Flipsen et al., 1995a). Ingested polyhedra dissolve in the alkaline environment of the midgut and virus particles are released into the gut lumen. After replication in the columnar epithelial cells, mainly budded viruses are formed, which spread infection to other susceptible tissues within the larval host (for reviews see Granados & Williams, 1986; Volkman & Keddie, 1990).

A detailed understanding of virus pathogenesis in insects remains to be elucidated, while much progress has been made by studying processes of virus gene expression in cell culture. In infected insect cells, AcMNPV genes are expressed in a coordinated fashion and can be subdivided into different temporal classes. Early genes, which are transcribed by host RNA polymerase II, are mainly expressed prior to virus replication and are thought to be involved in regulatory processes. Virus genes that are expressed during the following late and very late phases include those for structural proteins of both virus forms (for review see Blissard & Rohrmann, 1990).
Some of the early genes, such as ie1, ie2 (formerly IEN), pe38 and me53, have been shown in transient expression studies to be regulated exclusively by host factors, although these activating factors have not yet been identified (Guarino & Summers, 1986; Carson et al., 1988; Krappa & Knebel-Mörsdorf, 1991; Knebel-Mörsdorf et al., 1993). It is not known whether these genes are differentially regulated in larval tissues during infection and how their expression is linked to virus replication.

Previous studies have followed the progression of infection within insect larvae using AcMNPV recombinants expressing lacZ as a reporter gene under the control of the Drosophila melanogaster heat shock protein (hsp) promoter (Engelhard et al., 1994; Flipsen et al., 1995a). These recombinants allowed the determination of constitutive expression driven by an insect gene promoter but did not specifically reflect early virus gene expression. Therefore, we constructed AcMNPV recombinants with the lacZ gene under the control of the early promoters pe38 (AcMNPV/PE38) or me53 (AcMNPV/ME53). Since the pe38 and me53 genes are thought to be essential for virus replication, both recombinants carried the authentic early gene in addition to the early promoter–lacZ gene cassette.

By comparing reporter gene activity of AcMNPV/PE38 and AcMNPV/ME53 with the recombinant AcMNPV/HSP-p10 carrying two reporter genes, one driven by an insect gene promoter and the other by a very late virus gene promoter, we were able to discriminate between constitutive, early and very late virus gene expression in various tissues of Spodoptera exigua larvae. Our results demonstrated that expression of the pe38 and me53 genes is not tissue-specific. Furthermore, lacZ expression did not differ significantly under the control of early virus gene or constitutive promoters. β-glucuronidase (GUS) expression driven by the very late p10 promoter was observed in nearly all tissues except midgut goblet cells, Malpighian tubules and salivary glands. Interestingly, the me53 and pe38 genes were expressed strongly in salivary glands and Malpighian tubules, where virus replication probably does not occur.

### Methods

**Cells and viruses.** *S. frugiperda* IPLB21 cells (Vaughn et al., 1977) were grown as monolayer cultures at 27 °C in TC100 medium (Gardiner & Stockdale, 1975) supplemented with 10% fetal bovine serum. The AcMNPV isolates E (Tija et al., 1979) and E2 (Smith & Summers, 1978) were used as wild-type viruses. The biological activity of these strains showed no differences when compared in a bioassay according to Smits (1987). Infection with recombinant AcMNPV viruses was performed at a multiplicity of 10 p.f.u. per cell. Time zero was defined as the time when the inoculum was added to the cells (Krappa & Knebel-Mörsdorf, 1991).

**Recombinant viruses**

(i) AcMNPV/HSP-p10. The recombinant virus AcMNPV/HSP-p10 (Fig. 1) is based on the E2 strain of AcMNPV and has been described previously (Flipsen et al., 1995a). This recombinant contains two reporter genes, the Escherichia coli lacZ gene under the control of the constitutive hsp promoter of *D. melanogaster* and the *E. coli* GUS gene driven by the very late p10 promoter of AcMNPV.

(ii) *Construction of AcMNPV/PE38.* The recombinant virus AcMNPV/PE38 contains the lacZ gene under the control of the early pe38 promoter as an additional insert at the EcoRV site upstream of the polyhedrin gene (Gearing & Possee, 1990). The transfer vector pUC-PE38LacZ-rev was built as follows: the plasmid pBS LacZ was generated by isolating the BamHI-HindIII fragment of pAcDZ1, which includes the lacZ gene (Zuidema et al., 1990), and inserting this fragment into the vector pBluescript KS (+). The pe38 promoter fragment was generated by PCR amplification (Saiki et al., 1988) using plasmid pAcHind-F (Krappa & Knebel-Mörsdorf, 1991) as a template and the following primers: 5' TTTTTTCTCGAGATCTTAGTTTGTAGAGATGTACTGT 3' and 5' GGGGCTCGAGATTTTTTCAGGCCTCTTCGGTGCTC 3'. The spacer nucleotides (T)₅ and (G)₅ are followed by XhoI/BglII and XhoI sites, respectively. The 207 bp pe38 promoter fragment was inserted into the XhoI site of pBS LacZ. After excision, the pe38 promoter–lacZ gene cassette was inserted as a BamHI-BglII fragment into the BglII site of the pUC6/6/BglII plasmid (Weyer et al., 1990). The resulting pUC-PE38LacZ-rev construct includes the pe38 promoter in the opposite orientation to the polyhedrin promoter (Fig. 1).

(iii) *Construction of AcMNPV/ME53.* The recombinant AcMNPV/ME53 includes the lacZ gene under the control of the early me53 promoter upstream of the polyhedrin gene, and was produced in a comparable manner to AcMNPV/PE38. For PCR amplification of the me53 promoter fragment, plasmid pAcHind-G (Knebel-Mörsdorf et al., 1993) was used as a template with the following primers, which contain XhoI/BglII and XhoI sites, respectively, in addition to spacer nucleotides: 5' TTTTTTCTCGAGATCTTAGTTTGTAGAGATGTACTGT 3' and 5' GGGGCTCGAGATTTTTTCAGGCCTCTTCGGTGCTC 3'. The 202 bp me53 promoter fragment was combined with the lacZ gene and inserted into the pUC6/6/BglII plasmid as outlined above, thus producing pUC-ME53LacZ. This transfer vector contains the me53 and polyhedrin promoters in the same orientation.

(iv) *Transfection and screening for recombinant viruses.* AcMNPV/PE38 and AcMNPV/ME53 were obtained by co-transfection of virus DNA of AcMNPV plaque isolate E and the transfer vectors pUC-PE38LacZ-rev and pUC-ME53LacZ using the transfection reagent DOTAP (Boehringer Mannheim). The occlusion body-positive recombinant viruses were plaques-purified and analysed by Southern blotting. Cell culture-derived recombinant extracellular virus and polyhedra were obtained as described earlier (Flipsen et al., 1995a).

**Infection of larvae and histochemical analysis.** *S. exigua* larvae were obtained from a continuous laboratory culture as previously described (Flipsen et al., 1993). For histochemical analysis, early second instar larvae were inoculated orally with the AcMNPV recombinants by the droplet feeding method (Flipsen et al., 1993) using an inoculum of 10⁵ polyhedra per ml. About 10 larvae were dissected at 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 94 h after inoculation and subsequently fixed, washed and incubated for enzymatic detection of reporter gene expression (Flipsen et al., 1995a). The enzymatic detection gave rise to either a blue or red colour as a result of the presence of GUS and LacZ.
**AcMNPV pe38- and me53-driven lacZ expression**

Fig. 1. Schematic representation of the AcMNPV recombinants. In recombinant AcMNPV/PE38, the pe38 promoter–lacZ gene cassette including the simian virus 40 (SV40) transcription termination signal was inserted in the opposite orientation to the polyhedrin promoter. In recombinant AcMNPV/ME53, the me53 promoter–lacZ–SV40 construct was placed upstream of and in the same orientation as the polyhedrin promoter. For details of the cloning procedure see Methods. The orientations of the authentic genes pe38 and me53 are indicated by arrows. In recombinant AcMNPV/HSP-p10, the p10 coding region was been replaced by the GUS gene followed by the hsp promoter–lacZ gene–SV40 termination cassette (Flipsen et al., 1995a). Restriction site abbreviations: Ba, BamHI; Bg, BglII; H, HindIII; X, XhoI.

respectively. The preparations were then further fixed with a higher concentration of glutaraldehyde to maintain the ultrastructure. Subsequently the specimens were dehydrated, embedded, sectioned and studied using light and electron microscopy as described previously (Flipsen et al., 1993, 1995a). *S. exigua* larvae did not show any LacZ or GUS expression prior to or after AcMNPV infection.

**Infection of isolated organs.** For studies on susceptibility to infection, tissues such as salivary glands and the fat body were infected in vitro. Salivary glands and the fat body together with adhering tracheal elements were isolated from early fourth instar larvae of *S. exigua* and incubated with dispase for 20 min at 27 °C to remove the basal membrane. The organs were infected in TNM-FH medium (Hink, 1970) containing 10⁶ TCID₅₀ of AcMNPV/HSP-p10 per ml for 1 h at 4 °C. Subsequently, the tissues were washed three times and incubated in TNM-FH medium for 72 h at 27 °C.

**Immunogold labelling.** This was carried out as described previously (Flipsen et al., 1993). The polyclonal antiserum directed against alkali-liberated occluded virions primarily stained the structural glycoprotein gp41 (Smith & Summers, 1981; van der Wilk et al., 1987).

**Transcriptional analysis.** *S. frugiperda* cells infected with AcMNPV/ME53 or AcMNPV/PE38 were harvested at various times after inoculation to prepare cytoplasmic RNA. The 5' ends of the authentic virus transcripts and of the lacZ transcripts were mapped by primer extension analyses as previously described (Becker & Knebel-Mörsdorf, 1993). The analysis was performed with a me53-specific 28 base oligonucleotide primer (Becker & Knebel-Mörsdorf, 1993), a pe38-specific 29 base primer (Krappa et al., 1995) and a lacZ-specific 30 base primer (5' GTCACGACGTTGTAAAGCACGGGCCAGTGC 3').

**Results**

**Experimental design**

Our previous studies demonstrated that the pe38 and me53 genes belong to the major early transcribed genes (Krappa et al., 1992; Knebel-Mörsdorf et al., 1993). In this study, we investigated transcription of these early genes in vivo, whether it is restricted to specific larval tissues and whether activation of the genes is followed by virus replication. These questions were addressed by studying infection of *S. exigua* larvae with the recombinants AcMNPV/PE38 and AcMNPV/ME53, each of which carries an early promoter–lacZ gene cassette upstream of the polyhedrin gene, in addition to the authentic virus gene (Fig. 1). The sizes of the pe38 and me53 promoter fragments corresponded exactly to the functional pe38 promoter in the pPE38-CAT207 construct and the me53 promoter in plasmid pME53-CAT202, which were tested previously in transient expression assays (Krappa et al., 1992; Knebel-Mörsdorf et al., 1993).

**Transcriptional analysis of the pe38 and me53 promoters**

To investigate whether lacZ expression by the recombinants AcMNPV/PE38 and AcMNPV/ME53 mimics
Fig. 2. Primer extension analysis of pc38 transcription in *S. frugiperda* cells after infection with recombinant AcMNPV/PE38. Cytoplasmic RNAs (10 μg) prepared from uninfected cells (lane 0) or from cells at 3, 6, 12, 24, 48 and 72 h p.i. infected with AcMNPV/PE38 (lanes 3, 6, 12, 24, 48 and 72) were hybridized to a pe38-specific (a) or a lacZ-specific primer (b). The extended products were analysed on a 6% polyacrylamide gel. The extended products of 148 nucleotides for the authentic pe38 transcript and 136 nucleotides for the pe38–lacZ transcript are depicted by arrowheads. Positions of DNA size markers are shown on the left.

Fig. 3. Primer extension analysis of me53 transcription in *S. frugiperda* cells after infection with recombinant AcMNPV/ME53. Cytoplasmic RNAs (10 μg) prepared from uninfected cells (lane 0) or from cells at 3, 6, 12, 24, 48 and 72 h p.i. infected with AcMNPV/ME53 (lanes 3, 6, 12, 24, 48 and 72) were hybridized to a me53-specific (a) or to a lacZ-specific primer (b). The extended products of 85 and 124 nucleotides indicate the early and late starts of the authentic me53 transcript. The early and late me53–lacZ initiations are represented by the extended products of 136 and 176 nucleotides. For further details see Fig. 2.

Wild-type pc38 and me53 transcriptional initiation, the positions of the transcriptional start sites were determined. Cytoplasmic RNA was isolated from uninfected *S. frugiperda* cells or from AcMNPV/PE38- or AcMNPV/ME53-infected cells at 3, 6, 12, 24, 48 and 72 h p.i. and analysed by primer extension. The results of
transcriptional analysis in AcMNPV/PE38-infected S. frugiperda cells are shown in Fig. 2. Extension products of 148 nucleotides using the pe38-specific primer and of 136 nucleotides using the lacZ-specific primer were visible from 3 to 48 h p.i. (Fig. 2), confirming previous results on temporal pe38 transcription (Krappa et al., 1995). In addition, the levels of pe38 promoter-driven lacZ transcript did not decrease at 72 h p.i., which could be explained by higher mRNA stability compared to the authentic pe38 transcript. During the early phase of infection, pe38–lacZ transcripts were less abundant than wild-type pe38 transcripts (Fig. 2). Although the quantity of pe38–lacZ transcripts increased at 12 and 24 h p.i., the overall intensity of transcription was lower than in the wild-type. These results demonstrate transcriptional initiation occurred at the same site irrespective of whether the pe38 promoter was located at its authentic location or placed upstream of the very late polyhedrin promoter. Furthermore, the abundance of pe38–lacZ and authentic pe38 RNAs differed during the course of infection, which could be indicative of either modified regulation or a difference in RNA stability.
Table 1. Reporter gene expression in larval tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>hsp*</th>
<th>pe38 me53*</th>
<th>p10*</th>
<th>Ultrastructural observations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midgut cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>columnar</td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>Formation of virogenic stroma, virus particles, fibrillar structures and small, empty polyhedra</td>
</tr>
<tr>
<td>goblet</td>
<td>14</td>
<td>14</td>
<td>ND</td>
<td>No fibrillar structure or virogenic stroma formation, no polyhedra</td>
</tr>
<tr>
<td>regenerative</td>
<td>14‡</td>
<td>14</td>
<td>16</td>
<td>Normal polyhedra formation and empty polyhedra</td>
</tr>
<tr>
<td>Midgut muscle</td>
<td>16</td>
<td>16</td>
<td>60</td>
<td>Formation of single abnormal polyhedron with occluded nucleocapsids</td>
</tr>
<tr>
<td>Haemocytes</td>
<td>16</td>
<td>16</td>
<td>24</td>
<td>Polyhedra formation</td>
</tr>
<tr>
<td>Trachea</td>
<td>16</td>
<td>16</td>
<td>30</td>
<td>Polyhedra formation</td>
</tr>
<tr>
<td>Fat body</td>
<td>36</td>
<td>36</td>
<td>48</td>
<td>Polyhedra formation</td>
</tr>
<tr>
<td>Epidermis</td>
<td>48</td>
<td>48</td>
<td>60</td>
<td>Polyhedra formation</td>
</tr>
<tr>
<td>Malpighian tubules</td>
<td>48</td>
<td>48</td>
<td>ND</td>
<td>No fibrillar structure, virogenic stroma formation or nuclear hypertrophy, no polyhedra</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>48</td>
<td>48</td>
<td>ND</td>
<td>No fibrillar structure, virogenic stroma formation or nuclear hypertrophy, no polyhedra</td>
</tr>
<tr>
<td>Neural cells</td>
<td>60</td>
<td>60</td>
<td>72</td>
<td>Infection restricted to neural glial cells surrounding neurons, polyhedra formation</td>
</tr>
</tbody>
</table>

* Numbers represent h p.i. when lacZ expression (indicative of transcription from the hsp, me53 or pe38 promoters) and GUS expression (indicative of p10 transcription) were first detectable; ND, Not detected.
† Observations performed at 12 to 94 h p.i.
‡ Expression of lacZ was also observed at earlier times after infection due to direct passage of nucleocapsids.

The analysis of me53 transcription in AcMNPV/ME53-infected cells also revealed that initiation of the me53–lacZ and authentic me53 transcripts was comparable, as shown in Fig. 3. Primer extension studies of cytoplasmic RNA prepared from AcMNPV/ME53-infected S. frugiperda cells at 3, 6, 12, 24, 48 and 72 h p.i. suggested a switch from early to late transcriptional start sites at both the authentic and the additional me53 promoters. The same switch was observed previously in wild-type virus-infected S. frugiperda and TN-368 cells (D. Knebel-Mörsdorf, unpublished results). The precise mapping of the 5' ends showed an early transcriptional start 41 nucleotides upstream and a late start 82 nucleotides upstream of the predicted ATG start codon (data not shown). The early 5' end included a CAGT motif and the late start an ATAAG motif, which have been suggested to represent characteristic features of early and late/very late promoters, respectively (for reviews see Blissard & Rohrmann, 1990; Guarino & Smith, 1992). The abundance of early me53–lacZ and me53 transcripts was comparable, as shown by further primer extension studies, whereas late me53–lacZ transcription seemed to be less efficient (Fig. 3). Previously, a late 3-2 kb transcript was localized in the polyhedrin region that overlaps sequences upstream and downstream of the polyhedrin ORF in an antisense direction (Ooi & Miller, 1990). That study suggested that down-regulation of the 3-2 kb RNA occurred by transcription of the polyhedrin gene. The 3' end of the 3-2 kb RNA probably also overlaps the inserted me53–lacZ cassette in a sense orientation and the pe38–lacZ insertion in an antisense orientation (Fig. 1). Therefore, this late overlapping RNA could be a possible explanation for the decrease in late me53–lacZ transcription.

Infection of S. exigua larvae

To investigate early baculovirus gene expression, second instar S. exigua larvae were infected with polyhedra of the recombinants AcMNPV/PE38 and AcMNPV/ME53 and compared to infection with AcMNPV/HSP-p10. The detectable level of LacZ resulting from infection with AcMNPV/PE38 or AcMNPV/ME53 did not differ significantly. Early virus-driven lacZ expression was first observed in the midgut epithelium, mainly in columnar cells at about 3 h p.i. and at a lower frequency at about 6 h p.i. in midgut regenerative cells that were juxtaposed with infected columnar cells (Table 1). At 14 h p.i. reporter gene expression was also apparent in goblet cells neighbouring the primary infected midgut epithelial cells (Table 1). With increasing time the red
staining indicative of lacZ expression was observed in cells associated with the midgut basal membrane such as tracheal cells (Fig. 4a), muscle cells and haemocytes. Subsequently (36 h p.i. and later), early virus gene expression was detectable in distal tracheae, fat body, epidermis, major tracheae, skeletal muscle, neural glial cells, Malpighian tubules and in restricted zones of the salivary glands. Furthermore, at about 42 h p.i. lacZ expression could also be demonstrated in specific regions of the fore and hind gut which were in close contact with the tracheal elements. In Fig. 4(c) and (d), me53 promoter-driven lacZ expression is shown at 48 h p.i. in muscle cells, Malpighian tubules and fat body. Fig. 4(b) demonstrates strong lacZ expression in the Malpighian tubules at 72 h after AcMNPV/PE38 infection.

Similar results for lacZ expression under the control of the insect gene promoter were obtained following AcMNPV/HSP-p10 infection in S. exigua larvae (Flipsen et al., 1995a). In addition to lacZ, GUS expression driven by the very late p10 promoter was detected in midgut columnar and regenerative cells, midgut muscle cells, haemocytes, trachea, fat body, epidermis and neural glial cells at 48 to 72 h p.i. (Table 1) (Flipsen et al., 1995a). However, no late virus promoter-driven reporter gene activity was observed in midgut goblet cells, salivary glands or Malpighian tubules over the course of the experiment. At 92 h p.i. the experiment was ended because the tissues started to disintegrate and larvae began to liquefy.

These observations suggest that early me53 and pe38 transcription is not followed by very late viral transcription in salivary glands, Malpighian tubules and partly in midgut goblet cells as indicated by reporter gene expression. Furthermore, we screened for late virus gene expression by immunogold staining with a polyclonal antiserum detecting the structural protein gp41. Virus structural proteins were observed at 72 h p.i. in less than 5% of the lacZ-positive midgut goblet cells and in none of the lacZ-positive salivary glands and Malpighian tubules. The lack of late virus gene expression suggests...
virus replication does not take place in these tissues. Evidence supporting this conclusion also emerged from electron microscopy observations. Polyhedron formation, changes in the fibrillar structure and virogenic stroma formation were also investigated in comparison to early and late promoter-driven reporter gene activity (Table 1).

Minor quantitative differences in reporter gene expression from the various promoters were observed in different tissues. After infection with AcMNPV/PE38 or AcMNPV/ME53, stronger lacZ activity was observed in muscle cells, salivary glands and Malpighian tubules relative to other tissues and to lacZ expression under the control of the hsp promoter in these tissues. Whether these increased levels of LacZ were due to promoter or tissue specificity or to the absence of virus replication remains to be determined.

In vitro infection of salivary glands and fat body

As further evidence to support the lack of late virus gene expression as a tissue-specific effect even after an extended period of infection, salivary glands and fat body (as a control) were infected in vitro with recombinant AcMNPV/HSP-pl0. At 72 h p.i. no polyhedron formation, pl0 promoter-driven GUS expression or nuclear hypertrophy were observed in the salivary glands (Fig. 5a). At the same time, in vitro-infected fat body cells were filled with polyhedra (Fig. 5b) and showed very strong GUS expression.

Discussion

To provide new insights into the pathogenesis of AcMNPV in vivo, we constructed the recombinants AcMNPV/PE38 and AcMNPV/ME53 and analysed progression of infection in tissues of S. exigua larvae. This is the first study reporting on early viral transcription in infected larvae. Our results demonstrate the early genes me53 and pe38 are not expressed in a tissue-specific manner; indeed they are transcribed in nearly all larval tissues. Recently we identified SfNP-1, which recognizes the binding motif GATA in the pe38 and me53 promoters in S. frugiperda cells (Krappa et al., 1992). SfNP-1 binding does not contribute to basal promoter activity in cell culture. This observation led us to suggest that the GATA motif may be involved in tissue-specific expression of early virus genes (Krappa et al., 1992). While the observed lacZ expression indicates this is not the case, the possibility that transcriptional activity is modulated in the different tissues cannot be ruled out, since lacZ expression only indicates the onset of expression and gives no indication of the quantitative aspect of promoter strength.

A careful analysis of transcription from the inserted early promoter–lacZ gene cassettes was performed in cell culture to ascertain that lacZ expression represents specific early viral transcription. We observed that transcriptional initiation occurred at the same nucleotides as those at the authentic sites. These results are consistent with those of Dickson & Friesen (1991) who demonstrated no difference in the activity of the early 35K promoter compared to the polyhedrin promoter.

After infection of larvae with the recombinant viruses, lacZ expression was only indicative of early pe38 or me53 transcription, since the LacZ enzyme remains stable once it is made. Therefore, further late pe38 or me53 transcription could not be identified with these recombinants. It should be noted that there was no significant delay in the time course of me53- and pe38-driven lacZ expression compared to expression from the hsp promoter (Table 1).

Following infection of S. exigua larvae with any of the recombinant viruses, we observed that gene expression followed a specific temporal pattern in different tissues. Early virus gene expression was initially detected in the midgut columnar cells and subsequently in the regenerative and goblet cells, tracheae and haemocytes, fat body, Malpighian tubules and salivary glands and finally in the neural cells (Table 1). Analysis of the temporal expansion of virus infection in larvae of Trichoplusia ni and Heliothis virescens suggested the tracheal system is responsible for systemic infection within the insect (Engelhard et al., 1994; Washburn et al., 1995). These studies visualized gene expression in infected larval tissues using the recombinant AcMNPV-hsp/lacZ. In accordance with our observations, lacZ expression under the control of the hsp promoter was detected to some degree in the Malpighian tubules and salivary glands, which are closely connected with tracheoblasts (Engelhard et al., 1994; Washburn et al., 1995). However, the level of pe38- and me53-driven lacZ expression was much higher than from the hsp promoter in the Malpighian tubules and salivary glands. We extended these analyses by comparing early and late events of infection to discriminate between early virus gene expression and virus replication. Although virus replication took place in most larval tissues which showed lacZ expression, no virus pathogenesis-associated nuclear enlargement, virus stroma formation or production of virus structural proteins were observed in the Malpighian tubules or salivary glands. Keddie et al. (1989) have also observed that monoclonal antibodies directed against virus structural proteins rarely stained the Malpighian tubules of AcMNPV-infected Trichoplusia ni larvae. Therefore, it is likely that virus replication does not take place in essential organs like...
Malpighian tubules, salivary glands or probably midgut goblet cells. In conjunction with the recovery of the midgut epithelium, this would imply that the strategy of the virus is optimized to produce high quantities of progeny virus. This view is supported by recent observations that deletion of the AcMNPV ecdysteroid UDP-glucosyltransferase gene leads to increased speed of killing by AcMNPV, probably due to the early degeneration of the Malpighian tubules after infection (Flipsen et al., 1995b).

The mechanism responsible for the lack of late virus gene expression in certain tissues is unclear. Our initial findings with respect to tissue-specific transcription of early virus functions suggest that the presence of me53 and pe38 does not predict virus replication. No polyhedra were found in organs formed by the endoderm such as Malpighian tubules and salivary glands, and only a few or empty polyhedra could be detected in the midgut cells (Flipsen et al., 1993). Therefore, it is tempting to speculate that specific endodermal functions could be involved, at least in polyhedron formation, since polyhedra were only observed in tissues of ectodermal and mesodermal origin.

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References


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