Characterization of *Spodoptera exigua* multicapsid nucleopolyhedrovirus ORF17/18, a homologue of *Xestia c-nigrum* granulovirus ORF129

Wilfred F. J. IJkel, Els C. Roode, Rob W. Goldbach, Just M. Vlak and Douwe Zuidema

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

*Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) contains a number of genes with a homologue found so far only in a distantly related baculovirus. One of these, SeMNPV ORF17/18 (Se17/18) shares 55% amino acid similarity to ORF129 of *Xestia c-nigrum* granulovirus (XcGV). Se17/18 was transcribed in cultured *S. exigua* 301 cells, as a polyadenylated transcript of 1.1 kb. 5’-RACE analysis demonstrated that Se17/18 transcripts started at 134, 131 and 126 nt upstream of the putative translational start codon. These sites overlap with a baculovirus consensus early promoter motif. Se17/18 transcripts were detected by Northern blot analysis and RT–PCR with increasing abundance from 8 h to 24 h post infection (p.i.) and still present until 72 h p.i. A C-terminal GFP-fusion protein of Se17/18 was primarily localized in the cytoplasm of Se301 and Sf21 cells. A chicken polyclonal antiserum was raised that reacted specifically to Se17/18 protein produced in *E. coli*. However, no immunoreactive protein was detected in SeMNPV-infected Se301 cells and *S. exigua* larvae, neither in concentrated BV and ODV preparations. These observations and the inability to detect a C-terminal GFP-fusion protein of Se17/18 in Se301 cells using a GFP antibody suggest that Se17/18 protein is present, if at all, in spurious amounts. Based on the low homology of the Se17/18 protein to (methyl) transferases its possible involvement in transcription regulation is discussed.

**Introduction**

The beet army worm (*Spodoptera exigua*; Lepidoptera, Noctuidae) is an agriculturally important pest insect in (sub)tropical regions of the world and in greenhouses (Federici & Maddox, 1996). *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) is a highly pathogenic baculovirus for the beet army worm. SeMNPV differs from many other NPVs in that it is monospecific and highly virulent for *S. exigua* larvae (Smits, 1987). These biological characteristics make SeMNPV an attractive biological alternative for chemical insecticides. Detailed information on the expression and function of specific SeMNPV genes is important to gain further insight into baculovirus biology.

In previous studies, a mutant SeMNPV that lacked virulence in *vivo* was obtained within the first passage in cell culture (Heldens *et al*., 1996). On the basis of the entire nucleotide sequence of SeMNPV this mutant contained a contiguous 25 kb deletion encompassing SeMNPV ORFs15 to 41 (IJkel *et al*., 1999). So far, none of the 27 ORFs deleted has been experimentally shown to be essential for biological activity or virulence in *vivo*. Alternate cloning of SeMNPV, switching between cell culture and insects, and vice versa, resulted in mutants lacking ORFs15 to 28 (Dai *et al*., 2000). Of these, SeMNPV ORF17 (Se17) and ORF18 (Se18) were previously characterized as unique to SeMNPV (IJkel *et al*., 1999). Upon resequencing they appeared to be linked into a single ORF (Se17/18). Surprisingly, Se17/18 has a homologue in *Xestia c-nigrum* granulovirus (XcGV; Hayakawa *et al*., 1999), which is only very distantly related to SeMNPV. By computational analysis it appeared that Se17/18 has a cytoplasmic localization (Reinhardt & Hubbard, 1998). The function and significance of Se17/18 and its XcGV homologue are unknown. To gain more insight in the specific role of genes lost in random deletion mutations after propagation in cell culture, the functionality of Se17/18 was investigated by determining its transcriptional and translational activity as well as its subcellular localization.

**Methods**

- **Computer-assisted analysis.** Se17/18 (IJkel *et al*., 1999) and Xc129 (Hayakawa *et al*., 1999) were analysed using software on the
Fig. 1. For legend see opposite.
Plasmid construction. The complete coding region of Se17/18 including the stop codon was amplified by high fidelity ‘Expand’ long template PCR (Boehringer Mannheim) from cosmid 17 (Heldens et al., 1996) using primers containing 5’ BamHI and 3’ HindIII restriction sites and then cloned into pGEM-T. The plasmid was named pGEMSe17 and used for production of a gene-specific riboprobe. Plasmid pTriExSe17 was obtained by cloning the BamHI–HindIII fragment from pGEMSe17 into the BamHI and HindIII sites of the expression vector pTriEx-1 (Novagen). This plasmid was used for overexpression of the Se17/18 protein in E. coli.

To determine the localization of Se17/18 protein in insect cells, a GFP-fusion construct was made. The complete coding region of Se17/18, amplified as described above, was cloned into the BamHI and EcoRI sites of the previously described p166AcV5-GFP vector (Ijkel et al., 2000) and named p166Se17-GFP. Plasmid DNA was purified using Jetstar columns according to the manufacturer’s protocol (ITK Diagnostics). For each construct, the nucleotide sequence was checked using an automated DNA sequencer (Wageningen University, The Netherlands).

Cells, insects and viruses. The Spodoptera exigua cell-line Se301 (Hara et al., 1995), the S. frugiperda cell-line IPLB-Sf-21 (Vaughn et al., 1977) and the SeMNPV-US1 isolate (Gelernter & Federici, 1986; Munoz et al., 1998) were maintained and propagated as described previously (Ijkel et al., 2001). Haemolympid derived virus (first passage) was used to infect insect cells. A culture of S. exigua insects was maintained according to Smits & Vlak (1988).

BV and OVD preparations were purified from SeMNPV-US1-infected fourth-instar larvae of S. exigua, as described (Ijkel et al., 2001).

Total RNA isolation, Northern blot, RT–PCR, 3’ and 5’ RACE analyses. Total RNA was isolated from 2 × 10⁵ mock-infected and SeMNPV-US1-infected Se301 cells (m.o.i. 5 TCID₅₀ units per cell) at 4, 8, 16, 24, 48 and 72 h post-infection (p.i.). Total RNA was also isolated from fat body tissue obtained after dissection of six mock-infected and six SeMNPV-US1-infected S. exigua larvae (1.7 × 10⁴ polyhedra per larva) at 24, 48 and 72 h p.i. Cells and tissue were resuspended in 500 μl Trizol (GibcoBRL) and 100 μl chloroform, incubated for 8 min and centrifuged at 14 000 g for 15 min at 4 °C. The RNA in the water fraction was precipitated using isopropanol, centrifuged at 14 000 g for 10 min at 4 °C. Water was added with 70% ethanol and resuspended in 50 μl water. The RNA solutions were incubated at 55 °C for 10 min and quantified by absorbance at 260 nm.

For Northern analysis 8 μg of total RNA was electrophoresed in 1.5% agarose in the presence of glyoxal (Ausubel et al., 1994) and blotted onto a Hybond-N nylon membrane (Amersham). The Northern blot was hybridized with an Se17/18 messenger-specific riboprobe generated by SP6 polymerase with [α-³²P]CTP and BamHI-digested pGEMSe17/18. Fragment sizes were determined by staining the molecular mass marker (Promega RNA marker) with methylene blue after transfer onto the membrane.

RT–PCR was performed using the 5′/3′ RACE kit (Roche) with 2 μg purified total RNA as template per time-point. First-strand cDNA synthesis was performed using AMV reverse transcriptase and the oligo(dT) anchor primer according to the manufacturer’s instructions. The cDNAs mixtures were amplified by PCR using the PCR anchor primer and the gene-specific primer Se17/18SP1 (Fig. 1). The obtained PCR-products were analysed in 1:2% agarose gels.

The amplified RT–PCR products at 16 h and 24 h p.i. were used to determine the 3′ end of the Se17/18 in vitro mRNA, while the RT–PCR product at 72 h p.i. was used for the 3′ ends of the Se17/18 transcripts isolated from fat body tissue. All PCR-products were gel purified, cloned into pGEM-T and sequenced with 17 or SP6 primers.

The 5′ end of the Se17/18 transcript was determined using the 5′/3′ RACE kit (Roche) with 2 μg purified total RNA, isolated at 16 h and 48 h p.i. as template per time-point. Briefly, first strand cDNA synthesis was performed with the gene-specific primer Se17/18SP2 (Fig. 1). The cDNAs was purified with the High Pure PCR purification kit (Roche) and a poly(A) tail was added to the 3′ end using the terminal transferase with dATP. The tailed cDNAs were amplified by PCR using the oligo(dT) anchor primer and the nested gene-specific primer Se17/18SP3 (Fig. 1). A second PCR was performed using the PCR anchor primer and the nested primer Se17/18SP4 (Fig. 1). The PCR products obtained were gel purified, cloned into pGEM-T and sequenced with 17 or SP6 primers.

Production of polyclonal antibodies. Purified Se17/18 protein was prepared and isolated from E. coli BL21 cultures containing pTriExSe17/18, essentially as described (Ijkel et al., 2001). Purified Se17/18 was concentrated using Centricon-10 kDa filter devices (Amicon). Protein concentrations were determined with the Bio-Rad protein assay.

Two chickens were injected intramuscularly each with 12.5 μg purified Se17/18 protein using a water-in-oil adjuvant. The chickens were boosted after 6 weeks with 25 μg purified protein. Eggs were collected every day for 4 weeks and serum was collected 12 weeks after the boost injection. Western analysis using E. coli BL21 extracts expressing pTriExSe17/18 and PREP-cell purified Se17/18 was used to test the production of specific antisera.

Western analysis. Monolayers of Se301 cells were mock- or SeMNPV-US1-infected at an m.o.i. of 5 TCID₅₀ per cell. Cells were harvested at 4, 8, 16, 24, 48 and 72 h p.i., pelleted, resuspended in PBS and lysed in SDS–PAGE loading buffer by boiling for 5 min.

To analyse the presence of Se17/18 in larvae, 24 S. exigua third-star larvae were infected by contamination of an artificial diet with SeMNPV-US1 polyhedra (10 × LD₅₀). At 1, 2 and 5 days p.i. 12 larvae were harvested and the bodies frozen in 300 μl PBS. Protein samples were prepared by repeated freezing and thawing, and lysed with SDS loading buffer by boiling for 30 min.

To investigate if the Se17/18 protein is produced in insect cells, Sf21 cells (1 × 10⁷) were transfected with 2.5 μg of plasmid DNA, either p166Se17/18-GFP or p166-GFP (Ijkel et al., 2000), using Cellfectin
(GibcoBRL). Cells were harvested at 24, 30 and 48 h post transfection, resuspended and lysed.

All protein samples were separated by SDS–PAGE (Laemmli, 1970) and transferred to an Immobilon-P nitrocellulose membrane (Millipore) by semi-dry electrophoresis transfer (Ausubel et al., 1994). The membranes were incubated overnight in 10% block solution (Boehringer Mannheim) in PBS buffer at 4 °C. The membranes were allowed to react in PBS with Se17/18 antisera diluted 1:50,000, or polyhedrin antibody (derived from rabbit) 1:10,000 diluted or GFP antibody diluted 1:2000 (Molecular Probes) for 1 h at room temperature. After washing in PBS (three times 15 min), the membranes were incubated for 1 h at room temperature either with horseradish peroxidase (HRP)-conjugated rabbit anti-chicken immunoglobulin (Sigma) diluted 1:50,000 or with HRP-conjugated anti-rabbit diluted 1:5000 (Amersham) in PBS. After washing in PBS (three times 15 min) the signal was detected by ECL technology as described by the manufacturer (Amersham). The detection level of Se17/18 protein was determined by loading a serial dilution of purified baculoviral Se17/18 protein in SDS–PAGE loading buffer on SDS–PAGE-gel.

Fluorescence microscopy. Se301 cells (3 × 10⁵) and Sf21 cells (1 × 10⁶) were grown on glass cover-slips and transfected with 5 µg of plasmid DNA using Cellfectin (GibcoBRL). At 48 h post-transfection the cells were examined with a Zeiss LSM510 (confocal) laser scanning microscope for fluorescence using an excitation wave length of 488 nm and an emission band pass filter of 505–530 nm.

Results

SeMNPV ORF17/18

On resequencing ORF17 and ORF18 (IJkel et al., 1999), these ORFs appeared to form one contiguous ORF within the SeMNPV genome. The occurrence of an additional G, present after nt 20512, resulted in this correction, leading to one continuous ORF designated Se17/18. Se17/18 is located between nt 20121 and 20989 and encodes a putative protein of 289 aa with a predicted molecular mass of 33 kDa. Se17/18 is present in the reverse orientation in the genome relative to the polyhedrin gene (IJkel et al., 1999).

The region upstream of Se17/18 was analysed for the presence of possible transcription start sites, baculovirus consensus early CA(G/T)T and late DTAAG promoter motifs (Blissard & Rohrmann, 1989, 1990; Pullen & Friesen, 1995), downstream activating elements (Friesen, 1997) and host factor binding sites, such as GATA and CACGTG (Kogan & Blissard, 1994). Two early gene transcription initiation motifs (TATA-N₆-GGATT and TATAA-N₁₄-GACAGT) were found, respectively, 142 and 50 nt upstream of the putative Se17/18 translational start codon (Fig. 1). An additional TATA-box was located 117 nt upstream of the start codon, which could be linked to an AACATT sequence mapped 56 nt further downstream. A GATA host factor binding site was mapped 88 nt upstream of the putative ATG of Se17/18 (Fig. 1). A polyadenylation signal sequence (AATAAA) was identified starting at the last A of the TAA stop codon of Se17/18 (Fig. 1). The presence of putative promoter elements and a polyadenylation signal suggests that this ORF may be an active gene.

Northern blot and RT–PCR analysis of Se17/18 transcripts

The temporal regulation of Se17/18 transcripts was examined by Northern blot analysis and RT–PCR, using total RNA isolated from SeMNPV-infected Se301 cells. The Northern analysis revealed a band of 1·1 kb that could represent Se17/18 transcripts (Fig. 2A). These transcripts were detected at 4 h p.i., reached maximal transcription levels at 24 h p.i. and remained until 72 h p.i. The transcript size of 1·1 kb is in good agreement with the predicted ORF size of 870 nt for Se17/18. The size of the Se17/18 transcript suggests that it is most likely polyadenylated, assuming that one of the two putative early promoter sequences were used as transcription start sites. A second transcript of 3 kb was detected from 16 h p.i. onwards. This transcript could be a readthrough of Se17/18 into the cathepsin gene (Se16) and was more abundantly present than the 1·1 kb. Several transcripts larger than 6·6 kb were detected from 24 h p.i. onwards; their origins are unknown at present.

Using the more sensitive RT–PCR technique, further information was obtained about the temporal regulation of the 1·1 kb Se17/18 transcripts. A fragment of 790 bp was amplified (Fig. 2B) using a primer internal to Se17/18 (Se17/18SP1; Fig. 1) and the PCR anchor primer. The 0·8 kb RT–PCR fragment obtained appeared to be specific for Se17/18 on hybridization with an Se17/18-specific riboprobe (Fig. 2B). However, hybridization showed an additional Se17/18-specific band of 0·65 kb that was not clearly detected on agarose gel. Several attempts were made to clone this smaller fragment in pGEM-T, but none were successful. The 0·8 kb Se17/18 fragment was amplified from 8 h till 72 h p.i., with maximum abundance at 24 h and 48 h p.i. (Fig. 2B). Therefore, Se17/18 can be considered as a gene that is transcribed from early till late in infection.

To investigate if Se17/18 was also transcribed during SeMNPV in vivo infection in S. exigua larvae, RT–PCR was performed on RNA isolated from fat body tissue. An RT–PCR product was obtained at 72 h, but not at 24 h and 48 h p.i (Fig. 2C). Sequencing of two independent clones obtained after cloning this RT–PCR product into pGEM-T confirmed that the 0·8 kb RT–PCR product was derived from the Se17/18 sequence. Thus, Se17/18 was transcribed in infected insect cell cultures as well as during infection of insect larvae. Furthermore, the RT–PCR fragments obtained indicate that the Se17/18 transcripts contain a poly(A) tail, since the oligo(dT) anchor primer was successfully used to synthesize first-strand cDNA.

Transcriptional mapping of the 5’ and 3’ ends of the Se17/18 transcript

The 5’ end of the Se17/18 transcript was determined by 5’ RACE analysis using total RNA isolated at 16 h and 48 h p.i.

(To be continued)
from Se301 cells. A single cDNA was detected at both times tested. Three independent clones from each time-point were sequenced. The transcription start sites obtained varied for each time-point but are located within a small region. The most distal start site of Se17/18 transcription mapped at nt 134, while the most proximal start site was located 126 nt upstream of the ATG translation initiation codon, respectively, at the A in the sequence GGCATT and the second A in the sequence TCTAAT (Fig. 1). An intermediate start site mapped at nt 131 upstream of the putative translation start codon (Fig. 1). Thus, the Se17/18 transcripts initiated either immediately after an 8 nt region that contains a baculovirus consensus early promoter motif (Blissard & Rohrmann, 1990). The most distal start site of Se17/18 overlaps with the ATG start codon of the chitinase gene encoded on the complementary DNA strand. So, all Se17/18 transcripts presumably overlap with the major late chitinase transcripts, initiated from a baculovirus late promoter motif (ATAAG) located 13 nt upstream from its putative translation initiation codon (Fig. 1).

The 3’ end of the Se17/18 transcript was determined by sequencing the specific 0–8 kb RACE–PCR fragments obtained after RT amplification of total RNA purified from Se301 cells or S. exigua fat body tissue at various times p.i. The sequences obtained indicated that the 3’ ends of the transcripts isolated from cultured insect cells are identical to those isolated from fat body tissue and showed no variation in their poly(A) attachment sites. The 3’ end of the Se17/18 transcript was located 17 nt downstream of the stop codon at the last A in the sequence TAAT (Fig. 1). A conventional mammalian polyadenylation signal, consisting of an AATAAA motif and 20–30 nt downstream a diffuse (G)U-rich sequence, is located directly downstream of the stop codon. Hence, the Se17/18 transcript ended 12 nt downstream of the AATAAA motif. These results suggest that the conventional mammalian polyadenylation signal was used for the termination of Se17/18 transcripts in both Se301 and S. exigua fat body cells. Taken together, the data for the 5’ and 3’ end mapping predict a minimal transcript size of 1011 nt for Se17/18 [excluding the poly(A) tail]. The expected size is in good agreement with the 1–1 kb size determined by Northern analysis for the putative transcript of Se17/18 (Fig. 2A), assuming a poly(A) tail of approximately 100 nt.

**Localization of the Se17/18 protein in insect cells**

The subcellular localization of the Se17/18 gene product was investigated with a C-terminal GFP-fusion construct. The GFP-fusion construct was made in plasmid p166BRNX-AcV5.
Fig. 3. Localization of the Se17/18-GFP fusion protein in Sf21 and Se301 cells. Sf21 and Se301 cells were transfected with the control plasmid p166AcV5-GFP (panels A and B) or with plasmid p166AcV5-Se17/18GFP (panels C, D, E and F). At 48 h after transfection the cells were examined for fluorescence by confocal laser scanning microscopy. Phase contrast micrographs are shown to the right of the fluorescence micrograph. Overlay micrographs of the fluorescence and phase contrast micrographs are shown below the fluorescence micrographs.
Fig. 4. Western blot analysis of SeMNPV-infected Se301 cells (70,000 cells per lane), BV and ODV using a polyclonal antiserum against the Se17/18 protein. The corresponding times p.i. are indicated above the lanes (Mi, mock infected; Se17/18, 10 ng of purified Se17/18 protein). BV and ODV (10 µg per lane) represent isolated SeMNPV budded virus and alkali-disrupted occlusion-derived virus, respectively. The Se17/18 protein was identified using a chicken Se17/18 polyclonal antiserum and detected with a chemiluminescent substrate. Size standards are indicated in kDa and immunoreactive protein is indicated by an arrow.

(Ikel et al., 2000) and transcribed by the strong Orgyia pseudotsugata (Op)MNPV gp64 promoter. As a negative control, GFP alone, cloned in the same vector, was used. Sf21 and Se301 cells were transfected with 5 µg plasmid DNA, incubated for 48 h at 27 °C and subsequently examined for fluorescence by confocal laser scanning microscopy. The non-fused GFP protein showed homogeneous fluorescence in the cytoplasm and nucleus (Fig. 3A, B). The Se17/18 GFP-fusion protein, however, was mainly localized in the cytoplasm (Fig. 3C, D). This distinct pattern of fluorescence is consistent with the computer-predicted cytoplasmic localization of the Se17/18 protein (Reinhardt & Hubbard, 1998). Notably, the number of fluorescent cells after transfection with the Se17/18 GFP-fusion construct was considerably lower in Sf21 as well as in Se301 cells (<5%) than for the free GFP-control in these cell lines (30%). Furthermore, the majority of the cells transfected with the Se17/18 GFP-fusion construct and showing fluorescence were in bad condition, indicated by the shape of these cells (Fig. 3E). Some of these fluorescent cells even appeared to be apoptotic (Fig. 3F), in contrast to fluorescent cells in the negative control (data not shown). Cells first infected with SeMNPV and afterwards transfected with the Se17/18 GFP-fusion construct went into apoptosis and fluorescence was not detected (data not shown).

Immunodetection of the Se17/18 protein

The Se17/18 protein has a predicted molecular mass of 33.7 kDa. Antibodies were prepared by immunization of chickens with PREP-cell purified Se17/18 protein produced in E. coli. The chicken antiserum (polyclonal) obtained reacted strongly against this purified Se17/18 protein (Fig. 4, lane 17/18). Serial dilution of purified Se17/18 showed that 40 ng of protein was detectable using the chicken antiserum (data not shown).

Extracts of SeMNPV-infected Se301 insect cells, however, showed no immunoreactive protein when using this antiserum (Fig. 4). Furthermore, Se17/18 protein was also non-detectable in SeMNPV-infected S. exigua larvae at 1, 2 and 5 days p.i., while polyhedrin was clearly detected at 2 and 5 days p.i. using a polyhedrin antibody (data not shown). Also, concentrated BV and ODV preparations showed no immunoreactive protein using the chicken antiserum (Fig. 4).

To further investigate if the Se17/18 protein is produced in insect cells and is detectable with the Se17/18 antiserum, Sf21 cells were transfected with the Se17/18 GFP-fusion construct and examined by Western analysis. However, immunoreactive protein was detected neither with the Se17/18 antiserum nor with GFP antibody. In contrast, GFP protein was clearly detected in extracts of Sf21 cells transfected with the non-fused GFP construct (data not shown).

Similarity of Se17/18 to other (viral) proteins

Computer-assisted analysis was performed to elucidate if patterns of Se17/18 amino acid residues were also present in other (viral) proteins. Psi-BLAST searches (Altschul et al., 1997)
Fig. 5. For legend see opposite.
and Gap alignment revealed that the predicted Se17/18 amino acid sequence shares 55% similarity and 38% identity with XcGV ORF129 (Xc129; Hayakawa et al., 1999) (Fig. 5A). The conserved amino acid residues are equally distributed throughout the Se17/18 and Xc129 sequences. Most of their cysteine residues are conserved and primarily located at the C-terminal part of the proteins. Analysis of both predicted amino acid sequences did not reveal existing motifs for transmembrane regions, GPI-anchors or signal peptide sequences. BLAST searches revealed that the homology identified is also present at the nucleotide level and resembles the similarity found at the amino acid level.

A computer-assisted analysis was performed to elucidate whether patterns of residues conserved between Se17/18 and Xc129 were also present in other (viral) proteins. The N-terminal 100 aa of Se17/18 appeared to have low (∼25–35%) similarity to various proteins, such as the RNA-directed RNA polymerase (L protein) of Ebola virus [NP 066251], the histone acetyltransferase (Gcn5p) of Saccharomyces cerevisiae [NP 011768], the methylated-DNA protein cysteine methyltransferase (cmt) of Pseudomonas aeruginosa [AAG04384], the histidine decarboxylase (hdc) of Morganella morganii [AAA25321], the positive transcriptional regulator (Xys2) of Drosophila melanogaster [Q05092] and the DNA polymerase III α-subunit of Helicobacter pylori [P56157] (Fig. 5B).

The C-terminal part (aa 200–289) of the Se17/18 protein showed similarity (∼25–40%) to the small subunit of E. coli hydrogenase-2 (A55516), human papillomavirus type 82 E6 protein (NP 038151) and the Zyx102 protein of Drosophila melanogaster (AAF33231) (Fig. 5C). Notably, special conserved protein sequences (Fig. 5C). The C-terminal part of Se17/18 also showed similarity to the Lymnaea dispar (Ld)MNPV ORF53 (Ld53) protein (Kuzio et al., 1999). Ld53 is an homologue of Bombbyx mori (Bm)NPV ORF41 (Bm41), Helicoverpa armigera (Ha)SNPV ORF42 (Ha42), Autographa californica (Ac)MNPV ORF52 and SeMNPV ORF109 (Ayres et al., 1994; IJkel et al., 1999; Kuzio et al., 1999; Gomi et al., 1999; Chen et al., 2001). Strikingly, two of these four homologues, Ac52 and Se109, have smaller C termini and as a consequence lack the conserved cysteine residues present in Ld53 and Se17/18.

Most of the proteins that show (low) homology to the N-terminal as well as to the C-terminal parts of the Se17/18 and Xc129 proteins have two features in common; (i) they possess a transferase/reductase activity and (ii) they are involved in transcription. The middle part (aa 100–200) of the Se17/18 protein also showed low (∼20% amino acid similarity) homology to several proteins dedicated to various transferase activities (data not shown).

**Discussion**

In this study we report the temporal transcription, translation and subcellular localization patterns of the 17/18 protein of SeMNPV. Northern analysis showed that the amount of 1·1 kb Se17/18 transcripts increased from 4 h until 24 h p.i. and declined till 72 h p.i. (Fig. 2A). A similar transcription pattern was previously observed for other baculovirus genes, such as the AcMNPV pck/pml and lef4 genes, and the BmNPV bro genes (Durantel et al., 1998a, b; Kang et al., 1999). SeMNPV chitinase transcripts (Se19), which are present late in infection in abundance and overlap with the Se17/18 transcripts, may downregulate Se17/18 by antisense interaction.

Hybridization of the RT–PCR products revealed a second smaller band that reacted specifically with the Se17/18 riboprobe (Fig. 2B). This smaller product, and most likely favoured in PCR amplification, was still present in much lower amounts than the anticipated 0·7 kb RT–PCR product. Although it is possible that the smaller RT–PCR product is a PCR artefact, it cannot be excluded that it represents a 3′ truncated Se17/18 transcript.

Mapping of Se17/18 (Fig. 1) transcripts by 5′RACE showed that transcription initiated in a delineated region rather than at one specific nucleotide. This could either resemble the natural variation in Se17/18 transcription start sites or may be explained by RNA degradation at the 5′ end of the transcripts. Another explanation for the observed variation in the Se17/18 transcription start site could lie in a reduced or ‘slippery’ recognition of the GGCATT motif, since it is not completely consistent with the arthropod initiator cap site consensus, [A(A/C/T)CA(G/T)T] (Cherbas & Cherbas, 1993).

Comparison of the promoter regions of Se17/18 and Xc129 revealed no apparent nucleotide conservation at the Se17/18 transcriptional start sites (data not shown). The spacing of only 1 nt between the upstream TATA-box and the GGCATT transcription-initiation sequence of Se17/18 (Fig. 1) is very unusual (Roeder, 1991; Lu & Miller, 1995). Therefore, it is unlikely that this TATA-box is functional. Since no other
TATA-box is present in the 100 nt upstream of the Se17/18 transcription start site, its transcription could be TATA-independent.

Previously, TATA-independent transcription was reported for the OpMNPV gp64 gene (Kogan et al., 1995). Four elements, the host factor binding sites GATA and CACGTG, the transcription start site CA/G/TT and a CA-rich sequence in the 5’ untranslated region (UTR), were found to be required for TATA-independent transcription of gp64. Three of these, the transcription start site, the GATA sequence and CA-rich sequence are also present in the 5’ UTR of Se17/18, supporting the assumption that Se17/18 transcription is TATA-independent and may involve host factors.

Western analysis of protein extracts obtained from SeMNPV-infected Se301 cells and S. exigua larvae did not reveal an Se17/18 translation product (Fig. 4). This was quite unexpected since Northern blot analysis and RT–PCR demonstrated that transcripts were present at various times p.i. (Fig. 2). Concentrated BV and ODV samples also did not show any immunoreactive protein using chicken antiserum (Fig. 4). Our inability to detect the Se17/18 protein may be due to the low affinity of the Se17/18 antiserum and/or the low steady-state levels of Se17/18 protein during infection. The latter is more or less supported by the fact that (i) the Se17/18-GFP fusion protein also could not be detected with a commercially obtained GFP antibody and (ii) the very low percentage of insect cells that, on transfection with a construct designed to give high Se17/18-GFP protein expression, showed fluorescence. These facts, combined with the high mortality rate of cells transfected with Se17/18-GFP, may suggest that high concentrations of the Se17/18(-GFP) protein may exert a toxic effect on the insect cells. The observation that a mutant SeMNPV, obtained within the first passage in insect cell culture (Heldens et al., 1996), lacks Se17/18 among others suggests that the protein is non-essential in cell culture and may require a co-factor to become non-toxic.

The sequence similarity of Se17/18 (Fig. 5) to proteins that have in common transferase/reductase activities and of which some are known to be transcription regulators suggests that Se17/18 may have a function in transcription activation. A possible function of the Se17/18 protein may be the removal of methyl groups from methylated DNA sequences. If Se17/18 plays a role in transcription regulation this might also explain its toxic effect on insect cells, since its high expression level could distort the balance in host transcription. However, the cytoplasmic localization of the Se17/18 protein (Fig. 3; Reinhardt & Hubbard, 1998) does not support a direct role in transcription regulation, which is likely to take place in the nucleus of infected insect cells.

As the Se17/18 protein is highly homologous only (so far) to the Xc129 protein, both may play a role in specific virus–host interactions. Future studies in SeMNPV-infected larvae as well as the use of site-specific and null mutants with a recently constructed SeMNPV bacmid (Pijlman et al., 2002) will determine the significance of this gene in the SeMNPV infection cycle and may shed further light on its function.

This research was supported by The Netherlands Foundation for Chemical Sciences (CW) with financial aid from The Netherlands Organization for Scientific Research (NWO). The help of Angela Vermeesch with the tissue culture experiments and fluorescence microscopy has been highly appreciated.

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Received 12 April 2002; Accepted 16 July 2002