Expression of Viral DNA Polymerase Gene in Larvae of the Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), during Infection with Nuclear Polyhedrosis Virus

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Expression of viral DNA polymerase gene (*pol*) was examined in the midgut, fat body, silk gland and remaining carcass of larvae of the silkworm, *Bombyx mori*, infected with *B. mori* nuclear polyhedrosis virus (BmSNPV). Northern blot analysis detected a prominent viral *pol* transcript of approximately 3 kb and several other less prominent transcripts in these tissues. Mapping of the 5' end by RNase protection assay showed that the 3-kb *pol* transcript detected in the Northern blot analysis consisted of two species. These two species corresponded to the major and the largest transcripts, respectively, among seven viral *pol* transcripts identified previously in the BmSNPV-infected BM-N cells, demonstrating no viral *pol* transcripts unique to the infected tissues. Expression of each transcript varied considerably among tissues and with postinfection time. The abundance of overall *pol* transcripts coincided with those of DNA polymerase protein, viral DNA, viral polypeptides and polyhedrin in these tissues, with the exception of the carcass. These results suggest that the expression of viral *pol* transcripts is regulated differentially among tissues and during infection. They also suggest a possible role of viral early gene expression or viral DNA synthesis in the regulation of late and very late gene expression.

Key words: silkworm larva, nuclear polyhedrosis virus, DNA polymerase, gene expression, *Bombyx mori*

INTRODUCTION

Bombyx mori nuclear polyhedrosis virus (BmSNPV), the type species of the family Baculoviridae subgenus single nucleocapsid NPV (FRANCKI et al., 1991), is an infectious agent that causes acute disease in the silkworm, *B. mori*. This virus contains a double-stranded, covalently closed circular DNA genome of approximately 125 kbp (IATROU et al., 1985; IZUKA et al., 1987; MAEDA and MAJIMA, 1990) which is associated with a highly basic (arginine-rich) protein with a molecular weight (MW) of approximately 7,900 (7.9K) (MAEDA et al., 1991). The majority of BmSNPV nucleocapsids are singly enveloped to form rod-shaped virus particles or virions consisting of at least 80 structural polypeptides, as identified by two-dimensional high-resolution electrophoresis (SUGIMORI et al., 1990). The virions are embedded within a crystalline matrix or polyhedron, the major structural component of which is a single protein, designated as polyhedrin, with an approximate MW of 31K (IATROU et al., 1985; SUGIMORI et al., 1990).

In cultured cells infected with baculoviruses, viral gene expression has been shown to be regulated as an ordered cascade at the level of transcription, and viral genes are currently divided into four classes according to the time and manner of expression: immediate

early, delayed early, late, and very late (cf., FRIESEN and MILLER, 1986). Immediate early and delayed early genes are transcribed before initiation of viral DNA replication by an α -amanitin-sensitive RNA polymerase, apparently host RNA polymerase II (FUCHS et al., 1983; HUH and WEAVER, 1990). Immediate early genes, which largely encode regulatory proteins for the expression of later genes, are independent of prior expression of any viral genes, whereas the expression of delayed early genes requires one or more regulatory proteins previously expressed from immediate early genes. Late genes, most of which encode viral structural proteins, are transcribed concomitant with or after replication of the viral genome by an α -amanitin-resistant RNA polymerase (FUCHS et al., 1983; HUH and WEAVER, 1990). The transcription of very late genes begins well after the onset of late gene transcription, and continues until a very late postinfection time when most late genes have ceased transcription. Little is known of baculovirus gene expression in the tissues of infected insects.

Baculovirus DNA replication is an essential prerequisite for the production of progeny virions, and seems to be mediated directly or indirectly by the expression of several early viral genes. The expression products of these early viral genes presumably responsible for the baculovirus DNA replication include a DNA helicase (LU and CARSTENS, 1991), a proliferating cell nuclear antigen (PCNA)-like protein (CRAWFORD and MILLER, 1988; O'REILLY et al., 1989), and a DNA polymerase (TOMALSKI et al., 1988; BJORNSEN et al., 1992; CHAEYCHOMSRI et al., 1995). Although direct evidence has not been demonstrated, the fact that a unique DNA polymerase with distinct properties from those of cellular DNA polymerases is induced in baculovirus-infected cell culture and whole insects (KELLY, 1981; MILLER et al., 1981; WANG and KELLY, 1983; MIKHAILOV et al., 1986) strongly suggests an essential role of virus-encoded DNA polymerase in baculovirus DNA replication. BmSNPV DNA polymerase gene (*pol*) as with *Autographa californica* NPV (AcMNPV) *pol* has been shown to lack canonical TATAA and CCAAT elements in the upstream region and initiate transcription at multiple sites in cultured cells (TOMALSKI et al., 1988; CHAEYCHOMSRI et al., 1995). It has also been demonstrated in BmSNPV and AcMNPV that cycloheximide, an inhibitor of protein synthesis, does not inhibit, but delays, the expression of *pol*, whereas aphidicolin, an inhibitor of nuclear DNA replication, lowers the steady-state level of *pol* transcripts markedly (TOMALSKI et al., 1988; CHAEYCHOMSRI et al., 1995). These findings in cultured cells indicate that the gene expression of baculovirus *pol* is regulated by a complex and unique mechanism.

In the present study, we examined baculovirus *pol* expression in the fat body, midgut, silk gland and remaining carcass from silkworm larvae infected with BmSNPV. Accumulation of viral DNA and viral polypeptides was also examined in infected tissues to monitor the overall changes in the BmSNPV virion components during virus infection. Transcriptional analyses showed that among seven species of viral *pol* transcripts identified in BmSNPV-infected BM-N cells (CHAEYCHOMSRI et al., 1995), only two species were expressed in infected tissues. It was also shown that expression of these two *pol* transcripts occurred in a tissue-specific manner.

MATERIALS AND METHODS

Insect and virus. The silkworm, *Bombyx mori*, used was a hybrid race between Kinshu and Showa, and larvae were reared aseptically on an artificial diet (Kyodo Shiryō, Yokohama, Japan) at 25°C. A plaque-purified isolate, N9, of BmSNPV (NAGAMINE et al.,

1989) was used. The polyhedra for inoculation were propagated in BM-N cells as previously described (NAGAMINE et al., 1991), and partially purified by washing several times with distilled water (cf., KOBAYASHI and NAKAGAKI, 1984). The partially purified polyhedra (2.5×10^8 in 5 ml of sterile water) were applied to the surface of the artificial diet (area approximately 180 cm² and wet weight approximately 100 g), and 40 newly ecdysed fifth instar larvae were fed for 24 h at 23°C on the artificial diet contaminated with polyhedra. Control larvae were fed on an artificial diet to which the same volume of sterile water was applied. Although the optimal temperature of the development of nuclear polyhedrosis has been shown to be around 30°C (KOBAYASHI and CHAEYCHOMSRI, 1993), infected and uninfected larvae were incubated at 23°C to allow the infection to proceed slowly. The mortality of the infected larvae from nuclear polyhedrosis was 58 and 100% at 7 and 8 days postinfection (pi), respectively; surviving larvae were used for analysis at 7 days pi.

Isolation of cellular RNA and Northern blot analysis. Cellular RNA was extracted from tissues by guanidinium thiocyanate solubilization (CHOMCZYNSKI and SACCHI, 1987), and poly(A)⁺ RNA was selected with Oligotex-dT30 (Roche) according to the manufacturer's instructions. Five micrograms of poly(A)⁺ RNA was denatured and electrophoresed on a 0.8% formaldehyde-denaturing SeaKem agarose gel (FMC Corp.). The RNA on the gel was transferred onto a Hybond-N⁺ nylon membrane (Amersham) and hybridized with a ³²P-labeled BmSNPV DNA *pol* probe under the conditions described by SAMBROOK et al. (1989). The DNA probe was prepared from the 2.2-kbp *Pst*I-*Sma*I fragment of the *pol* (cf., CHAEYCHOMSRI et al., 1995) and labeled with [α -³²P]dCTP (Dupont NEN) by the rediprime DNA labeling system (Amersham).

Isolation of total cellular DNA and slot blot analysis of viral DNA. Total cellular DNA was prepared from tissues according to the method of VAN BEEK et al. (1990) with slight modifications. Midgut, fat body, silk gland, and carcass were each dissected out from infected larvae at different times pi, and homogenized in 5 vol. (v/w) of ice-cold TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) using a Hitachi HG 30 homogenizer. The homogenates were centrifuged at $5,000 \times g$ for 3 min to precipitate cell debris, and proteinase K and SDS were added to the supernatants at final concentrations of 1 mg/ml and 0.25%, respectively. The suspensions were incubated overnight at 37°C, and then extracted three times with TE-saturated phenol, once with phenol/chloroform (1 : 1, v/v), and once with chloroform. DNA was precipitated with 0.1 vol. (v/v) of 3 M sodium acetate, pH 5.2, and 2.5 vol. (v/v) of cold ethanol. The precipitates were collected by centrifugation, dried and resuspended in TE. DNA samples containing a total of 200 ng DNA were blotted onto a Hybond-N⁺ membrane in a MilliBlot transfer apparatus (Millipore). The blots were hybridized to either ³²P-labeled BmSNPV genomic DNA (partially digested with *Sau*3AI) or the DNA *pol* probe previously prepared for the Northern blot analysis (see above).

RNase protection assay. Mapping of the 5' end of BmSNPV *pol* transcripts by RNase protection assay was conducted as previously described (CHAEYCHOMSRI et al., 1995). Briefly, poly(A)⁺ RNA from infected tissues and ³²P-labeled RNA probe ER3 were hybridized overnight at 45°C, followed by treatment with RNase solution as recommended by the manufacturer (Ambion). The resultant RNase-resistant fragments were analyzed by a sequencing gel and visualized by autoradiography. The radiolabeled RNA probe ER3 was prepared by the transcription of linearized pSP72 (Promega) containing the BmSNPV *pol* DNA fragment in the presence of [α -³²P]CTP. For transcription, the *in vitro* transcription system with T7 RNA polymerase (Promega) was used.

Expression of DNA polymerase fusion proteins and production of anti-DNA polymerase

antisera. The 927-bp *Pst*I-*Cla*I and 1039-bp *Eco*RI-*Cla*I DNA fragments of the *pol* (cf., CHAEYCHOMSRI et al., 1995) were each subcloned into the *Pst*I-*Hind*III and *Eco*RI-*Xba*I sites of the pMAL-c2 expression vector (New England Biolabs), to construct plasmids pXP10 and pXE4, respectively. Constructs of pXP10 and pXE4 were introduced into *Escherichia coli* XL1-Blue (Stratagene). Bacteria were grown at 37°C to an absorbance of 1.0 at 600 nm. The fusion product of maltose binding protein (MBP) and BmSNPV DNA polymerase protein was expressed by induction with isopropyl- β -D-thiogalactopyranoside (IPTG) at a final concentration of 0.3 mM. After 1 h of induction, the bacteria were harvested and purified by affinity chromatography with an amylose column (New England Biolabs). The purified fusion protein was emulsified in Freund's complete adjuvant and injected into mice for immunization.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western blot analysis. Protein samples solubilized in the gel loading buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol) were resolved on 10 or 12.5% SDS-polyacrylamide gels according to the method of LAEMMLI (1970). The gels were either stained with Coomassie brilliant blue or electroblotted with Horizblot (Atto, Tokyo) onto a nitrocellulose membrane (Advantec Toyo, Osaka, Japan) at room temperature for 70 min at a constant current of 200 mA. Western blots were incubated overnight with gentle rocking at 4°C with the anti-DNA polymerase antiserum at a 1 : 500 dilution, and then reacted with ¹²⁵I-labeled goat anti-mouse IgG (ICN Biomedicals). For identification of virus-specific polypeptides and polyhedrin, antisera against polyhedra-derived virions and polyhedrin, respectively, prepared in rabbits (KOBAYASHI et al., 1990) were used as primary antibodies. Antibody-antigen complexes were detected using ¹²⁵I-labeled goat anti-rabbit IgG (ICN Biomedicals). Immunoreactivity was visualized and quantified by an imaging analyzer (BAS 2000, Fuji Photo Film).

RESULTS

Viral pol expression during infection

Two sets of experiments were performed to analyze the viral *pol* transcripts in silkworm larvae infected with BmSNPV. In the first set, Northern blots of poly(A)⁺ RNA from the midgut, fat body, silk gland and carcass of BmSNPV-infected larvae were hybridized to the DNA probe from a specific region of the *pol*. As shown in Fig. 1, a major transcript of approximately 3 kb was detected in infected tissues. The size of the transcript detected in the present experiment was consistent with that of transcripts previously observed in BmSNPV-infected BM-N cells (cf., CHAEYCHOMSRI et al., 1995). Accumulation of 3-kb *pol* transcript was abundant in the fat body and carcass, less abundant in the midgut, and barely detectable in the silk gland. In the fat body, the *pol* transcript was first detected at 4 days pi, maximally present at 5 days pi, declined by 6 days pi, and remained at this level at 7 days pi, whereas the *pol* transcript in the carcass was first detected at 4 days pi, maintained constant until 6 days pi, and declined at 7 days pi. Expression of the *pol* transcripts in the midgut was first observed at 6 days pi at a low level and was still present at 7 days pi.

In addition to the 3-kb *pol* transcript, a less prominent BmNPV *pol* transcript of approximately 6 kb was present in the fat body, midgut and carcass at 6 and 7 days pi, whereas a *pol* transcript of approximately 4 kb was observed in the fat body and carcass. In the carcass, two very faint bands of *pol* transcripts of approximately 10 and 7 kb were also detectable (Fig. 1). Transcripts with sizes far smaller than 3 kb, that were detectable in the

Fig. 1. Northern blot analysis of the *pol* transcripts in BmSNPV-infected larval tissues. Poly(A)⁺ RNA (5 μ g) from the infected midgut (A), fat body (B), silk gland (C), and carcass (D) at the various times p.i. indicated above the lanes was separated on a formaldehyde-denaturing SeaKem agarose gel, blotted onto a nylon membrane, and hybridized with ³²P-labeled DNA probe derived from the *Pst*I-*Sma*I fragment of the BmSNPV *pol*. Arrow indicates the position of the major transcript of approximately 3 kb, whereas the arrowhead indicates the second major transcript of about 6 kb.

fat body, midgut and carcass, probably represent artifacts generated by degradation during the experiments.

In the second set of experiments, an RNase protection assay was performed to examine whether the initiation sites of BmSNPV *pol* transcripts differed among tissues and during infection. When the RNase protection assay was carried out using the ER3 RNA probe and the poly(A)⁺ RNA from the midgut, fat body, silk gland and carcass of BmSNPV-infected larvae, two protected fragments of 180 and 309 nucleotides (nt) in length were detected (Fig. 2), and increasing the exposure time of the autoradiography to 120 h (usually 48 h) did not increase the number of protected fragments. This was in contrast to seven protected fragments in BmSNPV-infected BM-N cells (CHAHEYCHOMSRI et al., 1995). The 180- and 309-nt fragments have been identified in BmSNPV-infected BM-N cells to be the major and the

Fig. 2.

largest transcripts which place the initiation sites at -119 (-119 transcript) and -334 (-334 transcript), respectively, relative to the translational initiation codon (CHAEYCHOMSRI et al., 1995). The number and initiation site of the BmSNPV *pol* transcripts in these tissues were confirmed by primer extension analysis (data not shown).

The kinetics of the accumulation of -119 and -334 transcripts differed among infected tissues and with the times pi. In the fat body, the -119 transcript was first observed at 4 days pi, increased at 5 days pi and declined from 6 to 7 days pi, whereas the -334 transcript was detected at a low level at 4 days pi, increased at 5 days pi, and remained constant until 7 days pi (Fig. 2). In the carcass, the -119 transcript was first observed at 3 days pi, abundantly expressed at 4 and 5 days pi, and declined to 7 days pi, whereas the -334 transcript was first observed at the same abundance as that of -119 transcript at 3 days pi, reached a maximum at 4 days pi, and remained at this level until 7 days pi. In the midgut, the temporal pattern of the -119 and -334 transcripts was similar. These two transcripts were both weakly detected at 5 days pi, and increased slightly by 7 days pi. In the silk gland, the -334 transcript was detected at 6 and 7 days pi only after prolonged exposure of the autoradiograph, whereas the -119 transcript was undetectable under the conditions utilized (Fig. 2).

Viral DNA polymerase protein accumulation during infection

To identify the viral DNA polymerase protein, protein samples from infected tissues were resolved on 10% SDS-polyacrylamide gels and probed with antiserum against BmSNPV DNA polymerase. The Western blot analysis detected, among several non-specific bands, a protein band that was not observed in the uninfected tissues (Fig. 3). This protein had an approximate MW of 125K, that was roughly comparable to the 115K of the BmSNPV DNA polymerase predicted from the open reading frame of *pol* (cf., CHAEYCHOMSRI et al., 1995), and was detected in common in the fat body, midgut and carcass at later stages of infection. Based on these observations, we thought it reasonable to conclude that the protein identified in the Western blot analysis was from the viral DNA polymerase. The DNA polymerase protein was first detected at 5 or 6 days pi in the midgut, fat body and carcass (Fig. 3). The accumulation of DNA polymerase protein increased steadily through 7 days pi in the midgut, while in the fat body, it increased to 6 days pi but decreased at 7 days pi. This decrease in DNA polymerase protein in the fat body coincided with the decrease in viral DNA (cf., Fig. 4), suggesting a selective disintegration of severely infected cells into the haemocoel. In the carcass, DNA polymerase protein remained constant at a low level until 7 days pi. No detectable amount of DNA polymerase protein accumulated in the silk gland (Fig. 3).

Fig. 2. Mapping of the 5' end of the *pol* transcripts in the BmSNPV-infected larval tissues by RNase protection assay. (A) Location of the probe used to map the 5' end of the transcripts. Probe ER3 derived from *in vitro* transcription was 5' end labeled at the *Xho*I site in vector pSP72 containing the BmSNPV *pol* DNA fragment. The position of the label is indicated by an asterisk. (B) to (E) RNase protection analysis of the 5' end of *pol* transcripts. Poly(A)⁺ RNA (2 µg) was prepared from BmSNPV-infected midgut (B), fat body (C), silk gland (D), and carcass (E) at the various times pi as indicated above the lanes. RNA was hybridized to the ER3 probe and subjected to digestion with RNase. The RNase-resistant products were resolved on a 5% polyacrylamide sequencing gel along with the undigested probe (lane P). Molecular weight markers are indicated on the left. The numbers and arrowheads indicate the size of the protected fragments. For technical reasons, the mobility of the 309-nt (a) and 180-nt (b) protected fragments in the 7 day pi lane of panel B was relatively slower than that observed in other lanes.

Fig. 3. Western blot analysis of DNA polymerase polypeptide in the BmSNPV-infected larval tissues. Samples of total proteins (50 μ g) from the midgut (A), fat body (B), silk gland (C) and carcass (D) at various times pi were resolved on 10% SDS-polyacrylamide gels, electroblotted onto nitrocellulose membranes, and reacted with anti-DNA polymerase antiserum prepared in a mouse. Antibody-antigen complexes were detected by 125 I-labeled goat anti-mouse IgG, and visualized by an imaging analyzer. The band of DNA polymerase protein is indicated by an arrowhead. The numbers above the lanes represent the days pi.

Viral DNA accumulation during infection

Viral DNA accumulation in infected tissues was analyzed by slot blot hybridization. Total cellular DNA from the midgut, fat body, silk gland, and carcass of the BmSNPV-infected larvae was blotted onto a nylon membrane and hybridized to a BmSNPV *pol*

Fig. 4. Slot blot analysis of viral DNA in BmSNPV-infected larval tissues. Total DNA (200 ng) from infected midgut, fat body, silkgland, and carcass at various times pi was spotted onto a nylon membrane and hybridized with ^{32}P -labeled BmSNPV *pol* DNA probe. BmSNPV genomic DNA of known amount was also spotted as a standard. The numbers on the left represent days pi.

probe. In the fat body, viral DNA was first detected at 4 days pi, reached a maximum at 6 days pi, and decreased slightly at 7 days pi (Fig. 4). In the midgut and carcass, viral DNA was detected at 6 days pi and increased slightly at 7 days pi, although the amount and rate of increase of the viral DNA in these tissues were significantly lower than those in the fat body. Viral DNA was detected in very low quantities in the silkgland at 6 and 7 days pi (Fig. 4).

Hybridization of the same DNA preparations to the probe from BmSNPV genomic DNA partially digested with *Sau3AI* yielded essentially the same results except that the intensity of the hybridization was higher than that of the *pol* probe (data not shown).

Viral polypeptides and polyhedrin accumulation during infection

Alterations of overall polypeptide patterns in BmSNPV-infected larval tissues were examined by SDS-PAGE. The gels stained with Coomassie brilliant blue showed that the protein profiles changed quantitatively and qualitatively in larval tissues during infection with BmSNPV (data not shown). To examine the accumulation of viral structural polypeptides in BmSNPV-infected tissues, samples identical to those used for SDS-PAGE analysis were resolved on parallel polyacrylamide gels and subjected to Western blot analysis with antiserum against BmSNPV virions. The Western blot analysis showed that a number of viral polypeptides accumulated in the infected tissues (Fig. 5). In the midgut, fat body, and carcass, polypeptides with approximate MWs of 89K, 78K, 68K, 56K, 51K, 39K, 16.5K and 16K were detected. Polypeptides of 91K, 58K and 40K appeared in the fat body and carcass, and a 22K polypeptide appeared in the midgut and fat body. Polypeptides of 37K, 23K and 21.5K were specifically detected in the fat body. A 16.5K polypeptide was the only viral polypeptide detected in the silkgland. Among the viral polypeptides detected in BmSNPV-infected tissues, polypeptides of 78K, 39K, 16.5K and 16K were the major polypeptides. A viral polypeptide with an approximate MW of 16.5K was first detected at 4 days pi in the fat body, at 5 days pi in the carcass and at 6 days pi in the midgut and

Fig. 5. Western blot analysis of virus-specific polypeptides in BmSNPV-infected larval tissues. Samples of protein (20 μ g) from the BmSNPV-infected midgut (A), fat body (B), silk gland (C), and carcass (D) at various times pi were resolved on 12.5% SDS-polyacrylamide gels, and subjected to Western blot analysis using anti-BmSNPV antiserum prepared in a rabbit as the primary antibody. The antibody-antigen complexes were detected with 125 I-labeled goat anti-rabbit IgG, and visualized by an imaging analyzer. The numbers on the left indicate MWs ($\times 10^{-3}$) of the marker polypeptides. The numbers and arrowheads on the right indicate approximate MWs ($\times 10^{-3}$) of the virus-specific polypeptides.

Fig. 6. Western blot analysis of polyhedrin in BmSNPV-infected larval tissues. Samples of protein (20 μ g) from the BmSNPV-infected midgut (A), fat body (B), silk gland (C), and carcass (D) at various times p.i. were resolved on 12.5% SDS-polyacrylamide gels, along with the purified polyhedrin (50 ng), and subjected to Western blot analysis by anti-polyhedrin antiserum. Arrow indicates the major band of polyhedrin. For other details, see the legend to Fig. 5.

silk gland (Fig. 5). The polypeptide of 39K which appeared at 5 days pi in the fat body and carcass, and at 6 days pi in the midgut is probably the major capsid polypeptide, based on its abundance and MW (SUGIMORI et al., 1990, 1991).

Polypeptides from BmSNPV-infected tissues were also analyzed by anti-polyhedrin antiserum. The Western blot showed that the major band of about 31K was detected in all tissues examined and increased with the time pi (Fig. 6). The smaller bands which reacted with anti-polyhedrin antiserum probably represent degradation products of polyhedrin.

DISCUSSION

Baculovirus DNA polymerase gene (*pol*) has been shown to express multiple species of transcripts in infected culture cells (TOMALSKI et al., 1988; BJORNSEN et al., 1992; CHAEYCHOMSRI et al., 1995). In BM-N cells infected with BmSNPV, we found that at least seven different transcripts, with a common 3' end, of approximately 3 kb in length are expressed from the BmSNPV *pol* (CHAEYCHOMSRI et al., 1995). These seven *pol* transcripts place their 5' ends at -119, -120, -122, -132, -133, -197 and -334 relative to the translational initiation codon, that locate within or in the immediate vicinity of motif 5'-GCGTGCT-3', 5'-AGAGCGT-3' or 5'-GGCGGTGG-3'. The kinetics of the expression of each *pol* transcript in the BM-N cells change strikingly during BmSNPV infection, suggesting that the transcription of these *pol* transcripts is regulated differentially in infected cells. It is not known whether such heterogeneity of *pol* transcription is of any significance in tissue-specific regulation of viral pol expression in tissues infected with BmSNPV.

In the present study, we chose the midgut, fat body, silk gland and remaining carcass, which mainly consisted of hypodermis and cuticle, and analyzed the viral *pol* transcripts and DNA polymerase protein in these tissues during BmSNPV infection. The rationale for choosing these tissues is that the response to the BmSNPV infection has been suggested to vary considerably among these tissues. Even though no detailed comparative study has been reported on the yields of progeny virions and polyhedra among the infected tissues, it is presumed that the fat body is the major tissue of virus multiplication, while the silk gland is not a main target of the virus, as evidenced by low production of polyhedra (ARUGA et al., 1957, 1963). A considerable amount of progeny virions is produced in the midgut, but only a few small polyhedra are formed (KANKE and IWASHITA, 1969).

Employing these tissues from BmSNPV-infected larvae, an RNase protection assay showed that among seven species of 3-kb viral *pol* transcript identified in BmSNPV-infected BM-N cells, only two species were expressed (Fig. 2). These two *pol* transcripts place their 5' end at -119 (-119 transcript) and -334 (-334 transcript) relative to the translational initiation site, and corresponded to the predominant and largest species, respectively, among those identified in the BmSNPV-infected BM-N cells (CHAEYCHOMSRI et al., 1995), thus demonstrating no viral *pol* transcripts unique to larval tissues. The -119 transcript initiates at the A present 2 nt downstream of the motif 5'-GCGTGCT-3', whereas the -334 transcript initiates at the third G within the second octanucleotide motif 5'-GGCGGTGG-3' that is arranged as a direct repeat (CHAEYCHOMSRI et al., 1995). Transcripts initiating within or in the close vicinity of motif 5'-AGAGCGT-3', that serves as the transcriptional initiation sites of viral *pol* in the BmSNPV-infected BM-N cells as well as in the AcMNPV-infected *Spodoptera frugiperda* (SF) cells (TOMALSKI et al., 1988; CHAEYCHOMSRI et al., 1995), were undetectable in the infected tissues. Kinetic evidence showed that in the fat body and carcass, the -334 transcript remained constant through 7 days pi, while the -119 transcript

decreased at 7 days pi, indicating that BmSNPV *pol* used both motifs 5'-GCGTGCT-3' and 5'-GGCGGTGG-3' until 6 days pi but favored the motif 5'-GGCGGTGG-3' at 7 days pi. In addition, the relative abundance of -334 transcript to -119 transcript is higher in the carcass than in the fat body and midgut (Fig. 2). These results suggest that regulation of the expression of 3-kb viral *pol* transcripts is different not only between cultured cells and larval tissues but also among tissues in an infected larva. Taking into account the circumstantial evidence that baculovirus *pol* is transcribed by host RNA polymerase II (FUCHS et al., 1983; HUH and WEAVER, 1990; GLOCKER et al., 1992), it is possible that the regulation of BmSNPV *pol* in the infected tissues is dependent on tissue-specific factor(s).

Besides the 3-kb viral *pol* transcripts, several viral *pol* transcript species larger than 3 kb were detected in considerable quantities in the midgut, fat body and carcass at 6 and 7 days pi (Fig. 1). In the fat body and carcass, the temporal pattern of the expression of these larger species is different from that of 3-kb transcripts that are detected at 4 days pi and maintained through 7 days pi, suggesting that the expression of 3-kb and larger species is regulated through different mechanisms in these tissues. Although such larger species have not been found in BmSNPV-infected BM-N cells (CHAEYCHOMSRI et al., 1995), a similar larger *pol* transcript of about 5–6 kb was observed in the AcMNPV-infected SF cells (TOMALSKI et al., 1988), indicating that expression of the larger species is not confined to larval tissues. It is possible that expression of the 5–6 kb transcript in AcMNPV-infected SF cells is negatively regulated by some factors of either viral or cellular origin that are produced *de novo* after virus infection, since the expression of 5–6 kb *pol* transcript is accentuated by treatment of AcMNPV-infected SF cells with a protein synthesis inhibitor, cycloheximide (TOMALSKI et al., 1988). Whether the larger *pol* transcripts present in the BmSNPV-infected *Bombyx* larvae have any special function in the production of viral DNA is unknown.

Analyses by Northern blot hybridization and RNase protection assay showed that accumulation of BmSNPV *pol* transcripts was prominent in the fat body and carcass, less prominent in the midgut, and minor in the silk gland (Figs. 1, 2). Such differences in the abundance of *pol* transcripts among tissues coincide with the amount of DNA polymerase protein accumulated in the midgut, fat body and silk gland, suggesting the regulation of *pol* expression at the transcriptional level in these tissues. In the carcass, however, only a very little DNA polymerase protein was detected, in contrast to the abundance of *pol* transcripts (Figs. 1, 2 vs. Fig. 3). This may suggest that the expression of *pol* transcripts in the carcass is regulated at a posttranscriptional level. Alternatively, it is possible that the conditions of Western blot analysis utilized successfully for the midgut and fat body are not adequate for detection of viral DNA polymerase protein in the carcass.

In agreement with the earlier indication that BmSNPV is polyorganotrophic and possesses the ability to multiply in almost all tissues (WATANABE, 1975), analyses in the present study showed that not only viral DNA but also viral structural polypeptides and polyhedrin were present in all tissues examined (Figs. 4–6). The analyses also demonstrated that the abundance of these viral components differed among tissues examined, with their accumulation being most extensive in the fat body, moderate in the midgut and carcass, and least extensive in the silk gland. In these tissues, except for the carcass, the difference in the abundance of viral structural components is compatible with the difference in the expression of both *pol* transcripts and DNA polymerase protein, suggesting a possible role of viral *pol* expression or viral DNA synthesis in the regulation of late and very late gene expression.

Finally, our analysis showed that a considerable amount of polyhedrin accumulated in the infected midgut (Fig. 6). This result is in contrast to the observations under light and

electron microscopy that polyhedra are rarely detectable in infected midgut cells which yield many progeny virions (KANKE and IWASHITA, 1969). Our result, thus, suggests that the morphogenesis of polyhedra rather than the production of polyhedrin is restrictive in infected midgut cells. A similar situation has been observed in the midgut of *Trichoplusia ni* larvae infected with *T. ni* NPV in which only a few polyhedra with a few or no virions are formed, in spite of active production of polyhedrin (ADAMS and McCLINTOCK, 1991). The lack of ability of the midgut to produce a generous amount of polyhedra may be related to a unique role of the midgut during *per os* NPV infection, that is, the midgut is the primary target of NPV infection and provides budded progeny virions responsible for the establishment of systemic infection in the insect host (KEDDIE et al., 1989).

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