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Tissue-specific expression of silkmoth chorion genes *in vivo* using *Bombyx mori* nuclear polyhedrosis virus as a transducing vector

(baculoviruses/follicular cells/choriogenesis/transcriptional control/RNA processing)

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ABSTRACT **A pair of silkmoth chorion chromosomal genes, *HcA.12–HcB.12*, was inserted into a baculovirus transfer vector, pBmp2, derived from the nuclear polyhedrosis virus of *Bombyx mori*. This vector, which permits the insertion of foreign genetic material in the vicinity of a mutationally inactivated polyhedrin gene, was used to acquire the corresponding recombinant virus. Injection of mutant silkmoth pupae that lack all *Hc* chorion genes with the recombinant virus resulted in the infection of all internal organs including follicular tissue. Analysis of RNA from infected tissues has demonstrated that the two chorion genes present in the viral genome are correctly transcribed under the control of their own promoter in follicular cells, the tissue in which chorion genes are normally expressed. The chorion primary transcripts are also correctly processed in the infected follicular cells and yield mature mRNAs indistinguishable from authentic chorion mRNAs present in wild-type follicles. These results demonstrate that recombinant nuclear polyhedrosis viruses can be used as transducing vectors for introducing genetic material of host origin into the cells of the organism and that the transduced genes are transiently expressed in a tissue-specific manner under the control of their resident regulatory sequences. Thus we show the *in vivo* expression of cloned genes under cellular promoter control in an insect other than *Drosophila melanogaster*. The approach should be applicable to all insect systems that are subject to nuclear polyhedrosis virus infection.**

Genetic transformation represents a powerful methodological tool that facilitates the analysis of models of gene regulation. Although significant progress has been achieved in a number of plant and animal models for which methodology for stable or transient *in vivo* expression of cloned genes has been developed, confirmation of regulatory models of insect gene function has been hindered due to a lack of such expression systems. Thus far, only three types of *in vivo* expression systems have been developed for insect species. Two of them result in stable genetic transformation of *Drosophila melanogaster* embryos and rely on the utilization of two transposable elements, *P* and *hobo* (1, 2). Because of their specificity, however, these systems are not applicable to other insect species. The third system, termed somatic transformation (3), relies on the direct injection of cloned gene sequences into *Drosophila* embryos and results in a transient expression of the injected genes in somatic cells. No comparable expression assays are available for any other insect systems.

A number of insects are subject to infections by various RNA or DNA viruses (for extensive reviews, see refs. 4 and 5). In principle, such viruses could be engineered into vectors for introducing host genetic material into these organisms. Nuclear polyhedrosis viruses (NPVs) or group A baculoviruses, are known to infect a large number of lepidopteran species, including the silkworm *Bombyx mori* (4–6). NPVs contain a supercoiled, circular, double-stranded DNA genome 80–200 kilobases (kb) long, which is packaged in an enveloped rod-shaped nucleoprotein particle. The structure of the viral particles and the existing variability in the size of various NPV genomes allow the prediction that it should be possible to incorporate into the latter considerable lengths of exogenous DNA without affecting their ability to function autonomously. This was confirmed through the development of NPVs as vectors for expressing foreign genes of various origins under the control of viral promoter elements, particularly under the control of the powerful promoter controlling the expression of the gene encoding polyhedrin, one of the major viral proteins expressed during the late stages of infection. The NPVs of the alfalfa looper, *Autographa californica*, and the silkworm, *B. mori* (BmNPV), have been used as prototype vectors for high level expression of proteins encoded by various cDNAs or intronless genes under polyhedrin promoter control (for review, see refs. 7–10). Meanwhile, the cloning of the polyhedrin gene from other NPVs, a prerequisite to the development of similar expression vectors, has also been reported (11–13).

NPV expression vectors can also be used for expressing intron-containing genes and the infected cells can process correctly the resultant primary transcripts into mature cytoplasmic mRNAs (14–16). Translation of the latter in the infected cells has yielded the encoded polypeptides that, in one case, were also shown to undergo correct posttranslational processing (16).

We have reported (17) the construction of a number of polyhedrin promoter-based expression vectors derived from BmNPV and have also demonstrated that vectors containing deletions that inactivate completely the polyhedrin promoter can be used for acquiring engineered viruses whose functional properties with respect to replication, assembly, and infectivity are indistinguishable from those of wild-type BmNPV. These transfer vectors were constructed on the premise that they could be used for obtaining host DNA-containing recombinant BmNPVs that could permit the *in vivo* expression of the host sequences in infected cells without transcriptional interference from the powerful polyhedrin promoter.

In this paper, we report on the utilization of one of these transfer vectors for generating a recombinant BmNPV containing a fragment of *B. mori* genomic DNA. This fragment harbors two chorion genes of late developmental specificity and their associated 5′ and 3′ flanking sequences. We demonstrate that upon infection of live silkmoth pupae, the two chorion genes are efficiently expressed in ovarian follicular cells, the tissue in which chorion genes are normally expressed. Transcripts derived from the transduced genes are also correctly and quantitatively spliced in follicular cells but

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not in fat body, other tissues of the abdomen such as muscles and ganglia, or nonexpressing tissue culture cells. The processed transcripts yield mature mRNAs of sizes indistinguishable from those of the corresponding authentic chorion mRNAs present in follicular cells during choriogenesis.

MATERIALS AND METHODS

Cells and Animals. Bm5 cells (18) and strain 703, wild-type, and Gr^B mutant silkmoths (19) were used throughout these studies. Silkmoth pupae were infected with recombinant BmNPV 6–8 days after spinning and sacrificed 3–4 days after infection. For pupal infections, tissue culture medium of infected Bm5 cells was used as inoculum. Viral titers in the medium ranged from 2×10^7 to 2×10^8 plaque-forming units per ml, and 5 μl of appropriate viral stock dilutions containing 10^4–10^6 plaque-forming units were injected into the pupal abdomens plus 2–7 μg of collagenase in a maximum volume of 4 μl (20).

Transfer Vector and DNA Insert. A 3.8-kb *Eco*RI fragment of *B. mori* genomic DNA containing the chorion gene pair *HcA.12–HcB.12* was excised by partial *Eco*RI digestion of plasmid sc4.150 (21). This fragment was blunt-end ligated into the unique *Xba* I site of transfer vector pBmp2 (17) and the recombinant virus BmNPV2/HcA.12-HcB.12 was plaque-purified from the viral progeny obtained from Bm5 cells co-transfected with DNA of the recombinant transfer vector and wild-type BmNPV as described (17).

Nucleic Acid Isolation and Analysis. Total cellular lysates were prepared from various tissues by cell solubilization in urea/SDS buffer as described (22). Follicular tissue was solubilized at a rate of 5–7 μl per follicle, fat bodies and other abdomen tissues (mainly muscle and nervous tissue, termed carcass) were solubilized at a rate of 250–350 μl per pupa, and Bm5 cells were solubilized at approximately 100 μl per 10^6 cells. Total RNA was purified from the whole cell lysates by CsCl centrifugation (23). Lysate aliquots (maximum 200 μl) were layered over a 0.8–0.9 ml of cushion of 5.7 M CsCl in 10 mM Tris·HCl, pH 7.5/1 mM EDTA and centrifuged at 12°C for 12–16 hr at 90,000 rpm in the TL100.2 rotor of a Beckman TL100 tabletop ultracentrifuge. RNA pellets were rinsed briefly with water, solubilized in water, precipitated with two volumes of ethanol, washed with 70% ethanol, air-dried, and dissolved in a minimum volume of water. Recoveries ranged from 0.2 to 1.0 μg of RNA per μl of cellular extract. For dot hybridizations, aliquots of the RNA were denatured by mixing with equal volumes of 14.8% (vol/vol) formaldehyde/12× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate, pH 6.9) and incubating at 65°C for 20 min. Denatured samples were immobilized on a nylon membrane (Zeta-Probe) using a vacuum manifold. For Northern hybridizations, 3 μg of RNA from infected or uninfected control follicular cells was resolved on methylmercury gels and hybridized as described (21) with the exception that formamide was omitted from the hybridization mixture.

Probes and Hybridizations. For gene *HcA.12*, the hybridization probe was a γ-^{32}P-labeled synthetic 27-mer oligonucleotide, complementary to a portion of the first exon of the gene (16). For gene *HcB.12*, a 0.48-kb *Pst* I–*Ava* I restriction fragment of sc13.150 (21) containing the sequence from position −102 (promoter region) to position +377 (second exon; see also map in Fig. 2) was used as a probe, after nick-translation in the presence of [α-^{32}P]dCTP. Hybridizatons were carried out at 75°C when using restriction-fragment probes or at 65°C when using the oligonucleotide probe. All hybridizations were carried out at a 0.3 M Na^+ equivalent as described (21).

Primer-Extension Analysis. Primer extensions were carried out as described (22) using 5 μg of total follicular RNA from control animals or equivalent quantities of RNA purified from various infected tissues or Bm5 cells. The 162-base-pair (bp) single-end-labeled primer for gene *HcA.12* has been derived by end-labeling a 0.45-kb *Sau*96I fragment of sc8.150 (21) with polynucleotide kinase in the presence of [γ-^{32}P]ATP, further digesting it with *Rsa* I, and isolating the 162-bp subfragment. The isolation of the 214-bp single-end-labeled primer for gene *HcB.12* and analysis of primer-extension products were as described (16).

RESULTS

Transduced Chorion Genes Are Correctly Transcribed and Processed in Infected Follicular Cells. A 3.8-kb fragment of *Bombyx* genomic DNA was inserted into the unique *Xba* I site of transfer vector pBmp2, which contains a partially deleted polyhedrin gene (residues −89 to +338; note that the polyhedrin gene is numbered according to the NPV convention—the first nucleotide of the translation initiation codon is +1 and the transcriptional start site is at position −48; ref. 17). As shown in Fig. 1, the genomic DNA encompasses two complete, divergently transcribed chorion structural genes expressed late in choriogenesis, *HcA.12* and *HcB.12* (21), their common 5′ flanking promoter sequence, 271 bp long, and about 1.2 kb and 0.83 kb of their 3′ flanking sequences, respectively.

After plaque purification, the corresponding recombinant virus, BmNPV2/HcA.12-HcB.12, was injected into the abdomen of female silkmoth pupae. Homozygous Gr^B mutant females (24) were used as recipients. The eggs of these animals lack completely a large group of chorion proteins, including the two high cysteine proteins encoded by genes *HcA.12* and *HcB.12*, due to a large chromosomal deletion that eliminates the corresponding structural genes (19, 25). These recipients provide a null background over which the expression of the two transduced chorion genes can be measured.

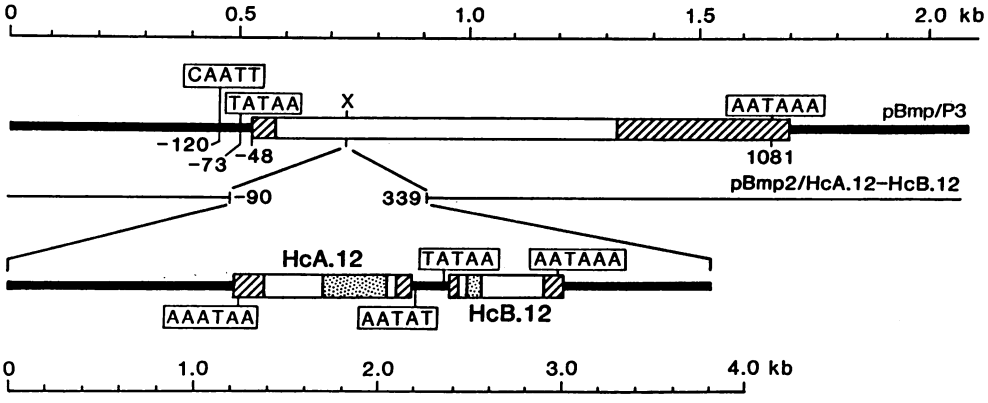

FIG. 1. Transfer vector structure. The two chorion genes (bottom line) were inserted into the unique *Xba* I site of vector pBmp2 (middle line). This vector was derived from clone pBmp/P3 (top line; ref. 18) containing the polyhedrin gene and encompasses a deletion of residues −89 to +338, which inactivates completely the polyhedrin promoter. Basal upstream regulatory elements and polyadenylylation signals are shown in boxes. Gene sequences are boxed. Untranslated regions are hatched, coding sequences are open, and intron sequences are stippled.

The presence of chorion RNA sequences in the follicular cells of infected pupae was analyzed by primer extension (Fig. 2). Extensions of 90 nucleotides (nt) or 572 nt would be predicted to result from correctly initiated HcA.12 transcripts (Fig. 2 *Left*), depending on whether the primer duplexes to mature (spliced) cytoplasmic mRNA or unspliced precursor. For gene *HcB.12*, corresponding primer extensions of 86 nt or 167 nt (Fig. 2 *Right*) would be expected. As can be seen from the results of these experiments, Gr^B mutant follicular cells infected with the recombinant virus (lanes IB) contain considerable quantities of correctly initiated and correctly spliced HcA.12 and HcB.12 mRNAs that are absent from the follicular cells of uninfected Gr^B mutant recipient controls (lanes B) but present in control wild-type follicles (lanes F). These specific transcripts are also absent from similarly infected Bm5 tissue culture cells (lanes C), which do not normally express chorion proteins. Because of the length of the intron sequence, it is difficult to deduce whether all HcA.12 transcripts are spliced. However, for HcB.12 mRNA, it is clear that splicing is quantitative. No extra bands can be detected at position +167 (arrowhead), and all other extension products are also present in the negative controls. We, therefore, conclude that the transduced genes are correctly transcribed in follicular, but not Bm5, cells under the control of their own regulatory elements and that primary transcripts are correctly and quantitatively spliced to produce functional mRNA. Densitometric quantitation of the extension products revealed that HcB.12 transcripts present in the infected cells (lanes IB) are on average 10 times more abundant than HcA.12 transcripts. This matches closely the relative abundance of 7 to 1 for these transcripts in wild-type follicular cells *in vivo* (lanes F; see also ref. 26) and suggests that the authentic relative strength of the common intergenic promoter region is maintained, even though the transduced genes are present as extrachromosomal copies. Alternatively, the observed differential in transcript abundance may be due to differences in RNA turnover rates.

The densitometric analysis of the primer extensions also revealed that the follicular RNA of infected pupae contained on the average 15 times fewer Hc transcripts than the RNA of wild-type follicular cells. This is due to the fact that viral infection of the follicular epithelium that surrounds each oocyte is not uniform, even in the presence of collagenase (20); therefore, not all follicular cells of infected animals contain virally transduced chorion genes. The follicular epithelium that surrounds each follicle consists of approximately 5000 cells (27). Wild-type follicular cells contain 15 *HcA* and *HcB* homologous genes, each of which is amplified 500- to 1000-fold due to endopolyploidization, which occurs during vitellogenesis (27). Under the hybridization conditions used, transcripts from all these copies hybridize with similar efficiencies to each probe. Thus, for wild-type follicular RNA, the primer-extension assays detect HcA and HcB transcripts derived from a total of $3.75–7.5 \times 10^7$ gene copies per follicle, presumably all of them active. In contrast, the extensions obtained with the RNA of infected Gr^B follicular cells, which are devoid of endogenous *Hc* genes, reflect the presence of transcripts derived from the virally transduced genes. In terms of transcriptionally competent copy numbers per cell, the latter are fewer than those present in wild-type follicles by at least one to two orders of magnitude (see *Discussion* for further quantitative details).

The nature of the cytoplasmic chorion transcripts derived from the transduced genes was also examined by Northern hybridization. The hybridization of follicular RNA obtained from a panel of infected Gr^B recipient pupae using a probe specific for one of the two transduced genes, *HcB.12*, is shown in Fig. 3. The infected follicular cells were found to contain variable quantities of hybridizing RNA sequences, whose electrophoretic mobility was identical to that of authentic HcB.12 mRNA. This documents that, in addition to correct transcription initiation and splicing, all other steps of posttranscriptional processing, such as endonucleolytic cleavage of the RNA precursors and polyadenylylation, are also correctly carried out by the infected cells (note the position of unspliced HcB.12 mRNA precursor in lane Ft). Hybridization of the same RNA preparations to a probe specific for HcA.12 mRNA yielded similar results (not

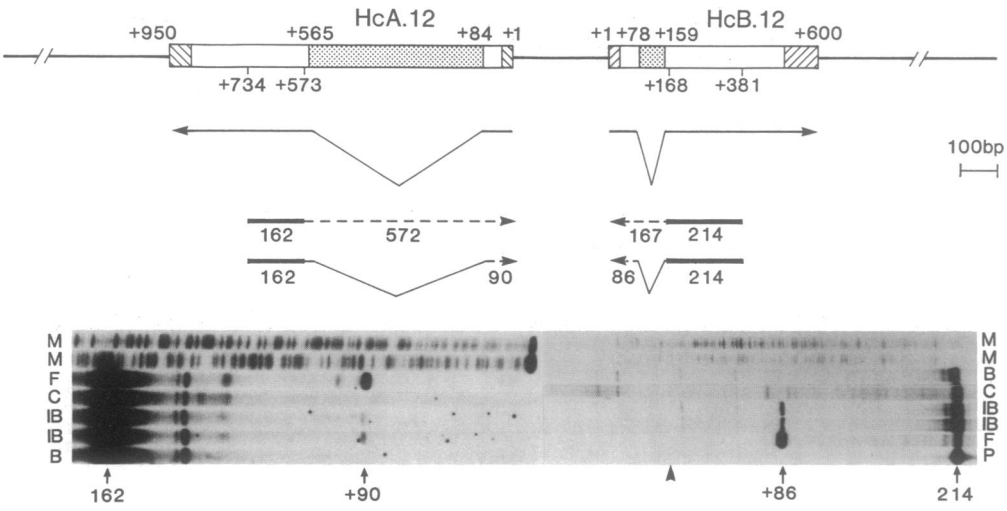

FIG. 2. Primer-extension analysis. Only the *Bombyx* genomic DNA insert containing the two chorion genes is indicated in the upper part of the figure plus their respective mRNAs. Gene region assignments are as in Fig. 1. Numbers above the genes indicate the transcriptional start sites, splice junctions, and the approximate 3′ termini of the two mature mRNAs. Numbers below the genes indicate the end points of the primers used in the extension analysis. The two primers (solid lines) and the predicted extensions on the two primary transcripts and mature mRNAs (broken lines) terminating at the authentic start sites (arrowheads) are depicted diagrammatically in the middle. The autoradiographic results of the electrophoretic analysis of the extension products obtained with each primer using total RNA extracted from control wild-type follicles (F), control Gr^B follicles (B), Gr^B follicles from two infected pupae (IB), and infected Bm5 tissue culture cells (C) are shown in the bottom part of the figure. Lane P contains the unextended primer for gene *HcB.12* and lanes M contain sequencing reactions serving as nucleotide chain length markers. Arrows and numbers indicate the positions and lengths of the two primers and their predicted authentic extension products obtained from wild-type follicular mRNA. The arrowhead on the right autoradiogram indicates the position where the +167 extension product derived from unspliced HcB transcripts would be expected to migrate.

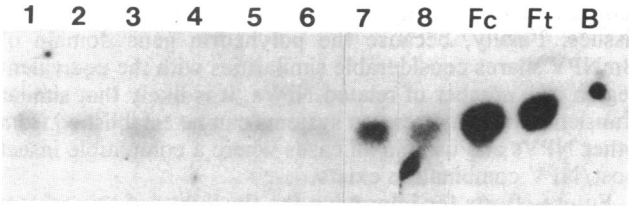

FIG. 3. Northern blot analysis of follicular RNA purified from infected Gr^B pupae. Total follicular RNA (3 μg) from eight infected Gr^B pupae (lanes 1–8) plus equivalent amounts of cytoplasmic (lane Fc) and total (lane Ft) wild-type follicular RNA and total follicular RNA of uninfected control Gr^B animals (lane B) was resolved on a methylmercury gel. After transfer, the immobilized nucleic acid was hybridized to the 0.48-kb *PstI–Ava* I probe, specific for gene *HcB.12*. The faint band above that of the mature mRNA in lane Ft is the result of the hybridization of the probe to unspliced HcB.12 mRNA that is present in the total follicular RNA preparation. The signal in lane B is an artifactual spot.

shown) except that the intensity of the hybridization was lower than that of HcB mRNA. Consistent with the primer-extension results (Fig. 2), the hybridizing RNA of infected follicles was found to be less abundant than that normally present in the follicular cells of wild-type animals.

Transduced Gene Transcription Is Developmentally Regulated. An additional set of experiments was carried out to find out whether the expression of the transduced genes is tissue-specific. Dot hybridizations of RNA from infected ovaries, fat bodies, carcasses (remaining abdomen tissues except for gut), and Bm5 tissue culture cells were carried out using a probe specific for HcB.12 mRNA. A representative set of results is shown in Fig. 4. The dot hybridizations (Fig. 4*A*) revealed that all cell types contained transcripts complementary to the probe. However, when the same RNAs were examined for the presence HcB.12 mRNA-specific transcripts by primer-extension analysis (Fig. 4*B*), correctly initiated and spliced HcB.12 transcripts were found only in follicular cells (lane IBF). No correct transcripts could be detected in any of the other cell types examined, although these cells obviously contain RNA species that result in nonspecific extensions (lanes IBf, IBc, and T). Ethidium bromide staining of the RNA after electrophoresis in denaturing gels (data not shown) revealed that the RNA preparations lacking specific HcB.12 transcripts had not been degraded during isolation. We, therefore, conclude that transcription of the chorion genes in infected animals is tissue-specific. The origin of the nonspecific hybridizing transcripts in cell types that do not normally transcribe chorion mRNA, presumably because they lack chorion-specific transcription factors, has not been further analyzed. We believe that these transcripts originate from random transcription initiation events occurring in the nonfollicular cells, such that only a small proportion of the aberrant transcripts can be detected by the probe used in primer-extension assays. Such nonspecific transcription has been shown to occur in Bm5 cells after transfection with cloned chorion genes (Y.A.W. Skeiky and K.I., unpublished results) and is particularly evident for the extension products derived from RNA of Bm5 cells infected with the same recombinant virus (Fig. 2, lane C, and Fig. 4, lane T), which revealed the presence of considerable quantities of transcripts of various lengths but complete absence of mature mRNA. The prominence of the hybridization signals obtained in the dot hybridizations of the nonfollicular RNA preparations is probably due to the fact that fat body, nervous tissue, muscle, and tissue culture cells, which are either covered by a relatively thin basal lamina or lack one completely, are infected by the virus more readily than follicular cells (20, 28, 29). Because of the presence of the thick basement membrane that completely surrounds each

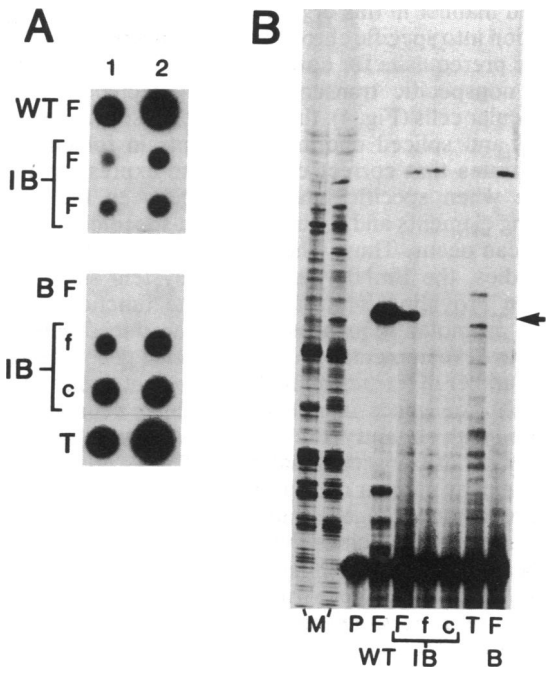

FIG. 4. Transcriptional specificity of transduced genes. (*A*) Dot hybridizations of immobilized RNA to the 0.48-kb *PstI–Ava* I probe, specific for gene *HcB.12*. Each dot contains 0.75 (left dots) or 2.25 μg (right dots) of CsCl-purified total RNA extracted from the following tissues: WTF, wild-type follicular cells; BF, uninfected control Gr^B follicular cells; IBF, infected Gr^B follicular cells (two different animals); IBf, infected Gr^B fat body; IBc, infected Gr^B carcass (mainly muscle and nervous tissue); T, Bm5 tissue culture cells. (*B*) Primer extensions for HcB.12 mRNA using the primer described in Fig. 3 and the same RNAs used in the dot hybridizations shown in *A*. Lane designations are as in *A*. Follicular RNA from only one of the two infected preparations of *A* was used. Lanes M contain sequencing reactions serving as markers. The arrow indicates the position of the authentic extension product obtained from wild-type cytoplasmic follicular RNA.

follicle, the latter are apparently infected by the recombinant virus only after a considerable lag: tissues such as fat body and carcass were found to contain large quantities of viral DNA 24 hr after inoculation with the virus, while viral DNA sequences could only be detected in follicular cells 36–48 hr after inoculation, and their abundance was significantly lower than that of the cells of other tissues (data not shown).

DISCUSSION

Our analysis has demonstrated that BmNPV can be used as a vector for transducing silkworm chromosomal genes into the cells of the organism. As exemplified by the analysis of the transcriptional properties of the chorion genes, the *in vivo* expression of the passenger genes is controlled by the resident cis regulatory elements and is tissue-specific. Although no attempt has been made to establish whether the transduced genes are also correctly regulated in a temporal fashion *in vivo*, we can predict that this will also prove to be the case. This prediction is based on studies that have demonstrated that activation of the two *Hc* genes under investigation is accompanied by the appearance of tissue- and stage-specific DNA binding activities, presumably transcription factors, in follicular nuclei (Y. A. W. Skeiky and K.I., unpublished data). Our results on the transcriptional specificity of the transduced chorion genes combined with the demonstration that silkmoth chorion genes introduced into various chromosomal domains of *D. melanogaster* by *P* element-mediated transformation are expressed in a tissue- and temporally

regulated manner in this organism (30, 31) suggest that gene integration into specific chromosomal domains may not be an absolute prerequisite for correct developmental control. Although nonspecific transcripts were detected in infected nonfollicular cells (Fig. 4), the exclusive presence of properly initiated and spliced chorion transcripts in follicular cells demonstrates that correct chorion gene expression is only possible when specific interactions between the resident cis-acting elements and tissue and stage-specific trans-acting factors can occur. Thus, when coupled to *in vitro* mutagenesis studies, the BmNPV expression system should prove useful in providing information on the functional role of specific promoter sequences of the transduced genes with respect to developmental control of chorion gene expression.

The number of transcriptionally competent copies of transduced chorion genes in infected follicular cells cannot be estimated with certainty, thus precluding precise quantitative assessments of transcriptional rates. This is due to the fact that gene copy number measurements in infected cells reflect the sums of unpackaged, transcriptionally active, viral genomes and silent genomes that occur as maturing or mature nucleocapsids destined initially for exit from the cells and later on, for the wild-type virus, for occlusion into polyhedra. The ratio of unpackaged to packaged viral genomes at any point of the infection cycle is unknown and, for this reason, only indirect calculations are feasible. Although as many as 2×10^4 mature wild-type nucleocapsids per cell are known to be occluded in nuclear polyhedra at the late stages of infection (8, 32), a number that matches the number of copies of *Hc* chorion genes normally present in the highly polyploid follicular cells, only a fraction of them is expected to represent transcriptionally active viral genomes at any given point of the infection cycle. Considering also that no more than 10–20% of the follicular cells of each follicle are infected by the recombinant virus even in the presence of collagenase (20), we infer that the total number of actively transcribed chorion genes in each infected follicle cannot exceed 2–5% of the number of *Hc* gene copies of a wild-type follicle. This is in close agreement with the observed abundance of Hc transcripts in infected follicles (Figs. 2 and 3) and suggests that the transcriptional competence of the virally transduced chorion genes is comparable to that of the endogenous (chromosomal) genes of wild-type follicular cells.

Although the BmNPV-derived expression system is a transient one (the host is killed by the virus 5–6 days after infection), it allows the study of the transcriptional properties of passenger genes over a period of up to 4 days. Because the transcribed molecules are also correctly processed in the infected cells, studies on posttranscriptional processing as well as translation of mRNA sequences and posttranslational processing of the resultant polypeptides should also be feasible. We have not yet conducted any protein labeling experiments to see whether any Hc polypeptides are present in the follicular cells or the chorion of infected pupae. However, our previous work (16) on the expression of the same genes under BmNPV polyhedrin promoter control in Bm5 tissue culture cells has established that chorion primary transcripts synthesized in these cells are correctly processed posttranscriptionally and translated efficiently into the corresponding polypeptides. These polypeptides are also quantitatively processed posttranslationally to produce mature chorion proteins indistinguishable from the authentic ones present in the extracellular chorion (16). These observations suggest that the two chorion polypeptides encoded by genes *HcA.12* and *HcB.12* should also be synthesized in the infected follicular cells.

Because infection of experimental animals can be carried at all stages of development and all tissues are subject to infection, the BmNPV system can be used for introducing any types of genes into the organism. Collagenase treatment, which was shown to be essential for efficient infection of the follicular cells (20), is not required for infection of other tissues. Finally, because the polyhedrin gene domain of BmNPV shares considerable similarities with the equivalent region of a number of related NPVs, it is likely that similar transient *in vivo* expression systems can be established from other NPVs and used in all cases where a compatible insect host/NPV combination exists.

Future efforts for improving the flexibility of this system should focus on two aspects: (*i*) the conditional or permanent inactivation of specific viral functions that occur after DNA replication, so that cell death can be prevented and (*ii*) the incorporation into the genome of such incapacitated viral vectors of specific host sequences that may facilitate homologous recombination and stable integration of the passenger genes into the cellular genome.

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