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Regulation of Expression of a Baculovirus Ecdysteroid UDPglucosyltransferase Gene

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The *Autographa californica* nuclear polyhedrosis virus *egt* gene encodes an ecdysteroid UDPglucosyltransferase which catalyzes the transfer of glucose from UDPglucose to ecdysteroid insect molting hormones. Expression of this gene allows the virus to block molting and pupation of infected insect larvae. In this study, we present the nucleotide sequence of the *A. californica* nuclear polyhedrosis virus *egt* gene and characterize *egt* gene expression at the transcriptional and translational levels. *egt* was transcribed as two 5′-coterminal mRNAs early in infection. Transferase activity was detected in infected cells and in the extracellular fluid by 3 h after infection. The majority of the activity accumulated in the extracellular fluid. We show that the *egt* gene product is a 60-kilodalton protein which is secreted from the infected cell. The *egt* gene is located in a region of the *A. californica* nuclear polyhedrosis virus genome which exhibited hypervariability in serially passaged virus stocks. Nucleotide sequence analysis revealed that the most common deletion occurring in these serially passaged virus isolates is located in the *egt* gene.

The *egt* gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV) encodes an ecdysteroid UDPglucosyltransferase which catalyzes the transfer of glucose from UDPglucose to ecdysteroids (19). Ecdysteroids are a family of steroid hormones essential for the induction of both larval-larval and larval-pupal molts in insects (9). AcMNPV infection of an insect larva blocks the ability of that larva to molt or pupate, through the expression of the *egt* gene. This is a unique example of the evolution of an insect virus to allow control of the infected host at the organismal level.

The *egt* gene product is related, at the protein sequence level, to several UDPglucuronosyltransferases (19), which catalyze analogous sugar transfer reactions in mammals (5, 23). Mammalian UDPglucuronosyltransferases are typically 50- to 60-kilodalton (kDa) membrane-bound proteins (8, 10, 13, 14) and are believed to catalyze conjugation reactions in the lumen of the endoplasmic reticulum of mammalian cells (13). However, our data suggested that the AcMNPV glucosyltransferase was secreted from the infected cell.

The region of the AcMNPV genome which includes the *egt* gene was first identified as an area which was mutated after serial passage of the virus in cell culture. Kumar and Miller (11) found that over half (335 of 667) of the virus isolates analyzed following serial passage of AcMNPV through *Trichoplusia ni* cells had a deletion of approximately 1 kilobase (kb) within the *Pst*I-G region of the genome.

In this report, we present the nucleotide sequence of the *egt* gene from wild-type (wt) and selected mutant viruses and show that the *egt* coding sequences are disrupted by the 1-kb deletion. In addition, we have characterized the regulation of the *egt* gene and gene product in cell culture. We show that *egt* is transcribed and translated early after infection. In agreement with our previous observations (19), we find that the enzyme is secreted as a stable 60-kDa protein. This suggests that *egt* action in ecdysteroid regulation might be exerted through its presence in the hemolymph of the infected insect.

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MATERIALS AND METHODS

Cells and viruses. *Spodoptera frugiperda* (fall armyworm) IPLB-SF21 cells (SF21) (25) were maintained in TC100 medium (GIBCO Laboratories, New York, N.Y.) supplemented with 10% fetal calf serum (GIBCO) and 0.25% tryptose broth. wt AcMNPV L-1 (12) was propagated and assayed on SF21 cells as described before (16). The mutant v1a7 was isolated following serial passage of AcMNPV through *T. ni* cells (11).

Cloning and sequencing. AcMNPV DNA *Pst*I to *Eco*RI (7.6 to 8.65 map units), *Bst*EII (8.35 to 8.7 map units), and *Eco*RI to *Sal*I (8.65 to 10.5 map units) fragments were cloned into the Bluescript plus and minus cloning vectors (Stratagene, San Diego, Calif.), using standard protocols (15). The appropriate fragment of the AcMNPV mutant v1a7 (11) was cloned in a similar manner. Overlapping nested deletions were generated from either end of the inserts by digestion with exonuclease III, using the protocol of Henikoff (7). Deletion clones were sequenced from the common primer sites in the cloning vectors, using the dideoxynucleotide chain termination procedure (22). Sequence data were compiled and analyzed by using the programs of Pustell and Kafatos (21) and Devereaux et al. (4).

Isolation and Northern blot analysis of RNA. SF21 cells (10^7/100-mm petri dish) were infected with AcMNPV at a multiplicity of infection of 20. For inhibitor studies, the cells were incubated in the presence of 100 μg of cycloheximide (Sigma Chemical Co., St. Louis, Mo.) ml^{-1} or 5 μg of aphidicolin (Sigma) ml^{-1}. Cycloheximide was added to the cells 30 min before infection and maintained in the tissue culture fluid throughout the infection, while aphidicolin was added to the cells following the 1-h virus adsorption period. At the appropriate times postinfection (p.i.), the cells were harvested and total RNA was prepared by guanidinium isothiocyanate lysis and centrifugation through cesium chloride (3). Polyadenylated [poly(A)$^+$] RNA was selected from total RNA by passage over an oligo(dT)-cellulose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) column (3). For Northern (RNA) blot analysis, 2 μg of poly(A)$^+$ RNA (or 20 μg of total RNA) from each time point was incubated in the presence of 2 M deionized glyoxal (Sigma)–10 mM

sodium phosphate for 1 h at 50°C. Following glyoxalation, RNA samples were electrophoresed through 1% agarose gels and transferred to Zetaprobe (Bio-Rad Laboratories, Richmond, Calif.) nylon membranes. A strand-specific probe (described in the text) was generated by using an appropriate exonuclease III deletion clone as single-stranded template DNA. The T3 primer, which is complementary to vector sequences flanking the insert, was annealed to the template DNA, and radiolabeled probe DNA was synthesized by T7 DNA polymerase (Sequenase; U.S. Biochemicals, Cleveland, Ohio) in the presence of [α-^{32}P]dCTP (Dupont, NEN Research Products, Boston, Mass.) and the other deoxyribonucleotides. The procedure typically yielded probe DNA labeled to 10^8 cpm μg^{-1} on one strand only. Hybridization of denatured probe (10^7 cpm) to RNA immobilized on Zetaprobe membranes was carried out by the instructions of the manufacturer.

Mapping of transcript termini. Specific DNA probes radiolabeled on a single strand at the 5′ or 3′ end were prepared by standard protocols (15). Details of the particular probes used are given in the text. The 5′ and 3′ ends of transcripts were mapped by using 30 μg of total RNA and 2,000 to 5,000 cpm of the appropriate probe, using the protocol of Weaver and Weissman (26). More precise location of 5′ ends was achieved by primer extension essentially as described previously (2). The primer used was a 17-mer oligonucleotide complementary to sequences downstream from the *egt* translational start site. Thirty micrograms of total RNA was used for each reaction, and the reaction was allowed to proceed for 30 min at 37°C. The same labeled primer was used to prime single-stranded DNA in a chain termination sequencing reaction as described above.

Construction of the vEGTZ recombinant virus. The *Pst*I to *Bam*HI (7.6 to 11.1 map units) fragment of the AcMNPV genome was inserted into the pUC19 cloning vector (28) to generate plasmid pUCBCPsB. This plasmid was cleaved within *egt*, using *Eco*RI (nucleotide [nt] 406) and *Xba*I (nt 1504), and the *Xba*I overhanging ends were filled in with T4 DNA polymerase. The *Escherichia coli* β-galactosidase gene (*lacZ*) was excised from plasmid pSKS104 (1) by cleavage with *Eco*RI and *Aha*III and inserted into pUCBCPsB. The resultant plasmid, pEGTZ, includes *lacZ* fused in frame to the first 87 amino acids of *egt* and lacks a substantial portion of the *egt* gene. The *lacZ* insertion is flanked by approximately 1.6 and 2.2 kb of viral sequences on either side, facilitating allelic replacement of the *egt-lacZ* fusion into the viral genome following cotransfection of pEGTZ and wt AcMNPV DNA. Recombinant plaques were selected by screening for β-galactosidase as described previously (20). The structure of the recombinant virus, vEGTZ, was verified by restriction enzyme analysis and Southern blotting experiments.

Analysis of protein expression. SF21 cells (10^6 per 35-mm petri dish) were infected with wt virus or vEGTZ at a multiplicity of infection of 20. At 5 h p.i., the cells were pulse-labeled with 25 μCi of [^{35}S]methionine (Dupont, NEN Research Products) in 0.5 ml of methionine-free TC100 medium for 1 h. The label was then removed and the cells were incubated in phosphate-buffered saline (12) for 1 h. The phosphate-buffered saline was harvested and used as the extracellular fraction, while the cells were lysed and processed as described before (18). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography were as described previously (18).

Ecdysteroid UDPglucosyl-transferase assays. SF21 cells (10^6 per 35-mm petri dish) were infected with wt AcMNPV or vEGTZ at a multiplicity of infection of 20. At the appropriate times p.i., the cells and overlying tissue culture fluid were harvested separately, and transferase activity was assayed as described before (19), using [^{3}H]ecdysone (Dupont, NEN Research Products) as substrate. Ecdysone and the ecdysone-glucose conjugate were separated by thin-layer chromatography on silica gel plates (19), and the radiolabel was visualized by fluorography.

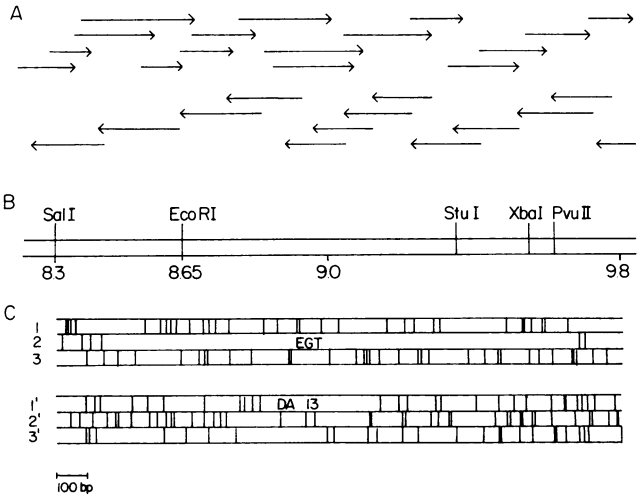


FIG. 1. Sequencing strategy and sequence of the AcMNPV *egt* gene. (A) Sequencing strategy used; (B) map of the region sequenced. Cleavage sites for relevant restriction endonucleases are shown, and the position of this sequence within the AcMNPV genome is indicated in map units. (C) Occurrence of stop codons in all six reading frames within this sequence. A stop codon is depicted as a vertical line. (D) Nucleotide sequence of this region, along with the predicted amino acid sequence of *egt*. Also shown are the endpoints of the deletion in v1a7 (arrows above the sequence indicate the terminal residues deleted). The 5′ end of the *egt* transcripts is indicated by a plus sign over the start site; # denotes the 3′ end of the 1.8-kb transcript. Potential transcriptional signals (TATA box, GTGT motif, and polyadenylation signal) are underlined with double lines. Putative N-linked glycosylation sites (N-X-S/T) are also underlined. The sequence data have been deposited in the GenBank data base (accession number M22619).

RESULTS

Nucleotide sequence of the *egt* gene. The sequencing strategy used to sequence the AcMNPV *egt* gene is shown in Fig. 1A. Two putative open reading frames (ORFs) (Fig. 1C) were identified in the sequence (Fig. 1D). The larger ORF is *egt*, which comprises 506 codons (Fig. 1D) and is predicted to encode a basic protein of 57,010 kDa (pI 9.83). *egt* proceeds in the clockwise sense on the AcMNPV genome. The other ORF (DA13) is within *egt* and proceeds in the opposite orientation (Fig. 1C). It is predicted to encode a highly basic (pI, 13.12) polypeptide of 116 amino acids. We have been unable to detect any mRNA species which would correspond to transcription in the counterclockwise sense (data not shown), and DA13 was not studied further. The sequence of part of this region has been reported previously by Guarino and Summers (6). We note that their sequence differs from the sequence presented here at nt 1524 (our coordinates). This residue is missing from their sequence. Loss of this base pair would shorten *egt* by 43 amino acid residues. While we are unsure of the basis of this discrepancy, we have sequenced this region several times in both directions and are confident of the sequence presented here.

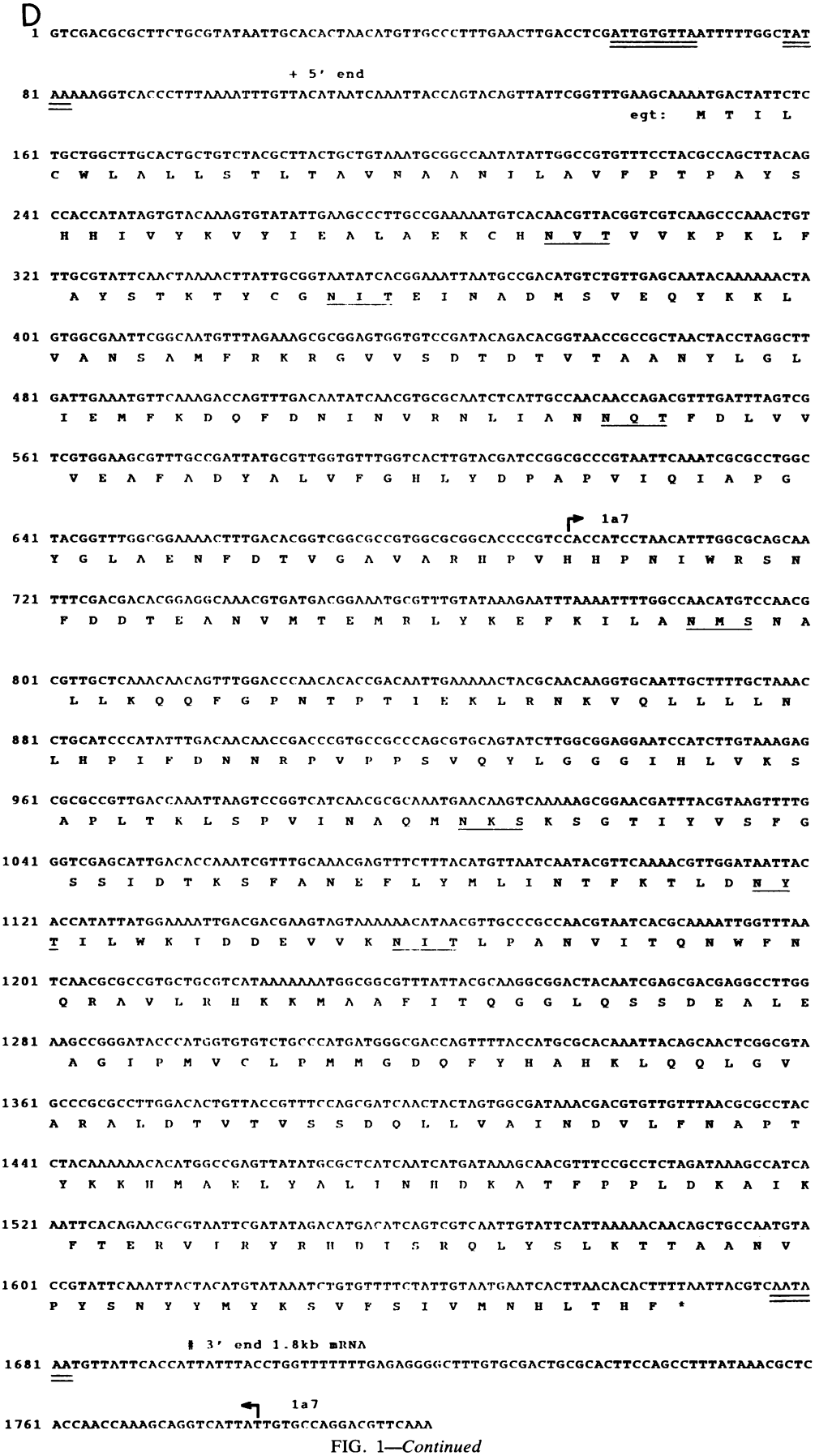


FIG. 1—*Continued*

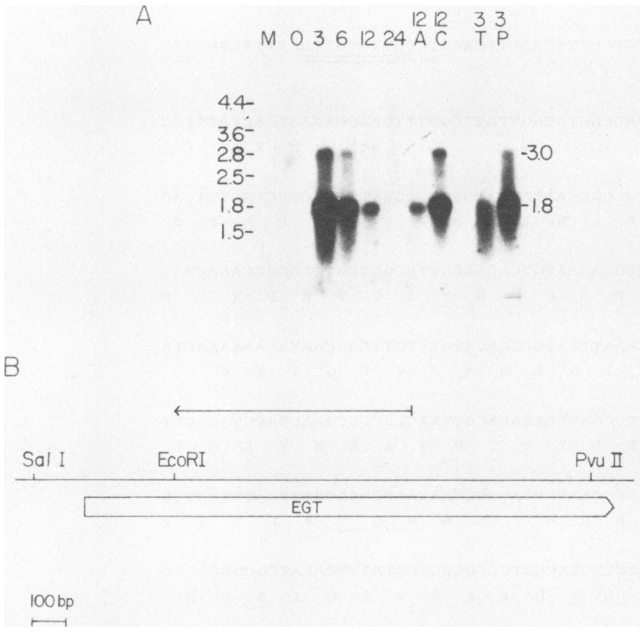


FIG. 2. Northern blot analysis of *egt* transcripts. Poly(A)$^+$ RNA was isolated from SF21 cells at various times (indicated in hours above panel A) after infection with wt AcMNPV. RNA isolated from mock-infected cells is designated M. Samples from cells treated with aphidicolin or cycloheximide were isolated 12 h p.i. and are in lanes 12A and 12C, respectively. A sample of total RNA was also prepared from cells infected for 3 h (lane 3T) and analyzed in parallel with poly(A)$^+$ RNA isolated at the same time (lane 3P). (A) Autoradiograph obtained after electrophoresis, blotting, and probing with a strand-specific probe. The sizes of the transcripts observed, and of the molecular weight markers, are given in kilobases beside the autoradiograph. (B) Probe used (thin arrow) relative to the *egt* ORF (open arrow). The direction of the arrow representing the probe shows the direction of the labeled strand.

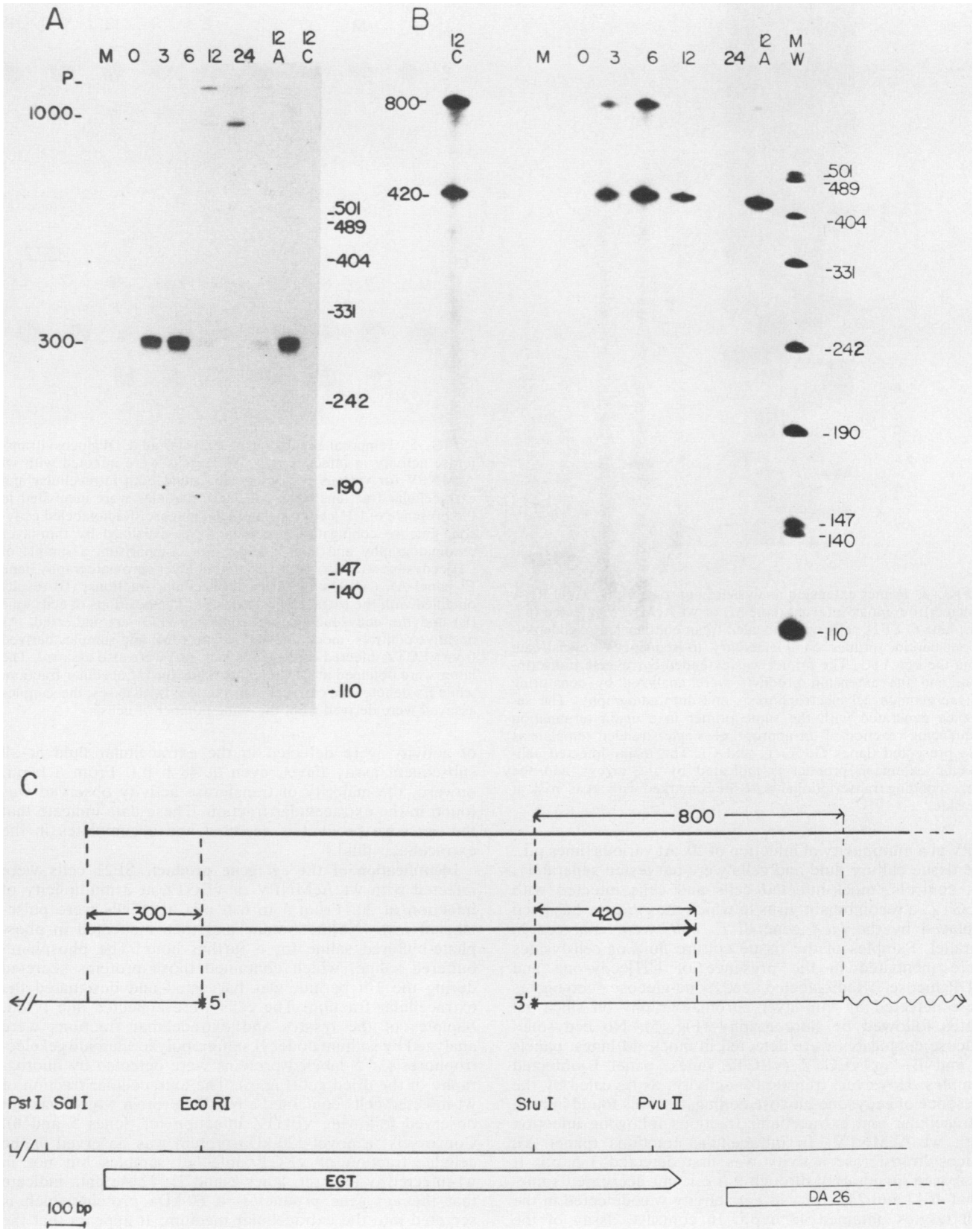
Regulation of *egt* transcription. The temporal control of *egt*-specific transcripts was investigated by Northern blot analysis of poly(A)$^+$ RNA isolated at various times p.i. of SF21 cells. Additional information on the temporal regulation of transcription was obtained by using the protein synthesis inhibitor cycloheximide, and the DNA synthesis inhibitor aphidicolin. For comparison, a sample of total RNA [not poly(A) selected] isolated 3 h p.i. was also analyzed. *egt* transcripts were detected with a DNA probe, extending from nt 406 to nt 1080, which was specifically labeled on one strand only (Fig. 2B). The results of this experiment are presented in Fig. 2A. Two *egt*-specific transcripts, of estimated sizes 1.8 and 3.0 kb, were observed. Synthesis of these transcripts was first observed 3 h p.i. Their levels decreased through 6 and 12 h p.i., and they were no longer detectable by 24 h p.i. Both of these RNAs were transcribed in the presence of aphidicolin and cycloheximide (lanes 12A and 12C), showing that their expression does not depend on prior viral protein synthesis or DNA replication. (The 3.0-kb transcript was clearly present in lanes 12, 12A, and 3T on the original autoradiograph.) These transcripts may therefore be classified as early transcripts. No transcripts were observed in the mock-infected control RNA (lane M). The pattern of transcripts detected with total RNA was qualitatively similar to that observed with poly(A)$^+$ RNA (lanes 3T and 3P), and total RNA was used in all further experiments.

Mapping the termini of *egt* mRNAs. Nuclease protection experiments were conducted to identify the 5′ and 3′ termini of the *egt*-specific transcripts. Total RNA isolated from SF21 cells at various times p.i. was annealed to a probe 5′-end-labeled at the *Eco*RI site at nt 411 or 3′-end-labeled at the *Stu*I site at nt 1275 (Fig. 3C). Hybrids were digested with S1 nuclease, and protected fragments were separated by denaturing acrylamide gel electrophoresis and visualized by autoradiography (Fig. 3A and B). The 5′-end mapping experiment (panel A) revealed a single major protected fragment of about 300 nt early after infection, corresponding to a start site around nt 110. The temporal regulation of this start site was in good agreement with that observed in the Northern blot experiments described above. A minor protected fragment of about 1,000 nt was also observed late in infection, demonstrating the existence of a minor late specific transcript initiating further upstream. This transcript was not investigated further. When the 3′-end-labeled probe was used (panel B), two protected fragments, of approximate sizes 420 and 800 nt, were observed. The 420-nt protected fragment would correspond to a 3′ terminus around nt 1695, which is 25 nt downstream from the *egt* stop codon. Protection of the 800-nt fragment indicated that some transcripts extended beyond the viral sequences present in this probe (Fig. 3). We have found that these transcripts extend beyond the sequences presented here and probably terminate within ORF DA18 (D. R. O'Reilly, A. L. Passarelli, I. F. Goldman, and L. K. Miller, submitted for publication). They correspond to the 3.0-kb mRNA species observed in Fig. 2.

The 5′ end of the *egt* transcripts was precisely localized by primer extension analysis. The primer, a synthetic oligodeoxynucleotide complementary to sequences 9 to 25 nt downstream of the *egt* ATG, was annealed to total RNA isolated from mock-infected or 6-h-infected SF21 cells and extended with reverse transcriptase. The extension products were electrophoresed alongside a sequencing ladder generated with the same primer (Fig. 4). Two infected cell-specific extension products were observed, which would correspond to initiation sites at nt 106 and nt 109 (Fig. 1D). It is possible that the latter site is an artifact due to premature termination of the reverse transcriptase.

Regulation of *egt* activity. To monitor the temporal regulation of *egt* activity, SF21 cells were infected with wt AcM-

FIG. 3. Nuclease protection mapping of *egt* transcripts. Total RNA isolated at various times after infection of SF21 cells with wt AcMNPV was annealed to 5′- or 3′-end-labeled probes and digested with S1 nuclease. Protected fragments were separated by denaturing gel electrophoresis and visualized by autoradiography. (A) Results obtained with the 5′-end-labeled probe; (B) results obtained with the 3′-end-labeled probe. Again, the times after infection at which the samples were isolated are indicated in hours above the autoradiographs. Lanes 12A and 12C denote samples obtained from cells treated with aphidicolin and cycloheximide, respectively, while M indicates the mock-infected control. The sizes of the protected fragments and of the markers are in base pairs. (C) Probes used, positions of the *egt* and DA26 ORFs, and principal transcripts in this region. The asterisk at the end of each probe denotes the labeled end, and the wavy line represents probe sequences derived from the plasmid vector. Overlaps between transcripts and probes (which correspond to the protected fragments observed in panel A) are illustrated.


A
M 0 3 6 12 24 12 A 12 C
P-
1000-
300-
-501
-489
-404
-331
-242
-190
-147
-140
-110
B
12 C
800-
420-
M 0 3 6 12 24 12 A M W
-501
-489
-404
-331
-242
-190
-147
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Eco RI
Stu I
Pvu II
EGT
DA 26
100 bp

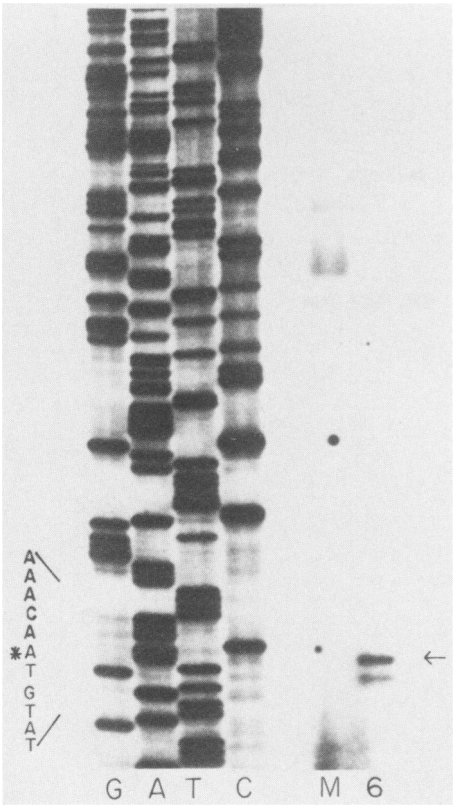

FIG. 4. Primer extension analysis of *egt* transcripts. Total RNA isolated from mock-infected (lane M) or wt AcMNPV-infected (6 h p.i.; lane 6) SF21 cells was annealed to an end-labeled oligodeoxyribonucleotide primer complementary to sequences downstream from the *egt* ATG. The primer was extended by reverse transcription, and the extension products were analyzed by denaturing polyacrylamide gel electrophoresis and autoradiography. The sequence generated with the same primer in a chain termination sequencing reaction of an appropriate single-stranded template is also presented (lanes G, A, T, and C). The major infected cell-specific extension product is indicated by the arrow, and the corresponding transcriptional start site is marked with an asterisk at the side.

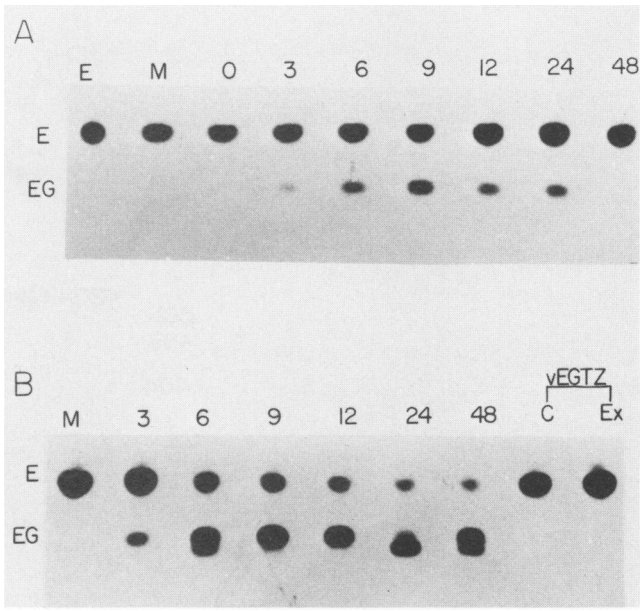

FIG. 5. Temporal regulation of ecdysteroid UDPglucosyltransferase activity in infected cells. SF21 cells were infected with wt AcMNPV for various periods of time, and both intracellular and extracellular fractions were collected. Samples were incubated in the presence of [^{3}H]ecdysone and UDPglucose. Radiolabeled ecdysone-glucose conjugation products were visualized by thin-layer chromatography and fluorography. For comparison, a sample of [^{3}H]ecdysone was also subjected to thin-layer chromatography (lane E, panel A). (A) Assays of the intracellular fractions; (B) results obtained with the extracellular fractions. The positions of ecdysone (E) and the ecdysone-glucose conjugate (EG) are indicated. As negative controls, mock-infected samples (M) and samples derived from vEGTZ-infected cells (which lack *egt*) were also assayed. The latter were obtained at 12 h p.i. C denotes the intracellular fraction, while Ex denotes the extracellular fraction. In all cases, the samples assayed were derived from the same number of cells.

NPV at a multiplicity of infection of 20. At various times p.i., the tissue culture fluid and cells were harvested separately. As controls, mock-infected cells and cells infected with vEGTZ, a recombinant virus in which the *egt* gene has been replaced by the *lacZ* gene of *E. coli*, were analyzed in parallel. Samples of the tissue culture fluid or cell lysates were incubated in the presence of [^{3}H]ecdysone and UDPglucose. Radiolabeled ecdysone-glucose conjugates were detected by thin-layer chromatography on silica gel plates followed by fluorography (Fig. 5). No ecdysone-glucose conjugates were detected in mock (M lanes, panels A and B)- or vEGTZ (vEGTZ lanes, panel B)-infected samples. However, transferase activity, as revealed by the presence of ecdysone-glucose conjugates, was found in both intracellular and extracellular fractions following infection with wt AcMNPV. In intracellular fractions (panel A), glucosyltransferase activity was first detected 3 h p.i. It increased moderately through 9 h p.i. but decreased somewhat at 12 and 24 h p.i. No *egt* activity was detected in the cell lysates obtained 48 h p.i. In contrast, assay of the extracellular medium (panel B) showed that transferase activity increased dramatically from 3 to 6 h p.i. High levels of activity were detected in the extracellular fluid at all subsequent assay times, even at 48 h p.i. From 6 h p.i. onward, the majority of transferase activity observed was found in the extracellular fraction. These data indicate that the *egt* gene product is secreted and accumulates in the extracellular fluid.

Identification of the *egt* gene product. SF21 cells were infected with wt AcMNPV or vEGTZ at a multiplicity of infection of 20. From 5 to 6 h p.i., the cells were pulse-labeled with [^{35}S]methionine and then incubated in phosphate-buffered saline for a further hour. The phosphate-buffered saline, which contained those proteins secreted during the 1-h period, was harvested and designated the extracellular fraction. The cells were collected and lysed. Samples of the lysates and extracellular fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. ^{35}S-labeled proteins were detected by fluorography of the dried gel (Fig. 6). The extracellular fraction of wt-infected cells contained a 60-kDa protein which was not observed following vEGTZ infection (cf. lanes 5 and 6). Conversely, a novel 120-kDa protein was observed in the cellular fraction of vEGTZ-infected samples but not in wt-infected lysates (cf. lanes 2 and 3). These data indicate that the *egt* gene product is a 60-kDa protein which is secreted into the extracellular medium. It appears that the *egt-lacZ* fusion protein is not secreted as efficiently.

Deletion of *egt* in serially passaged virus. The variant v1a7

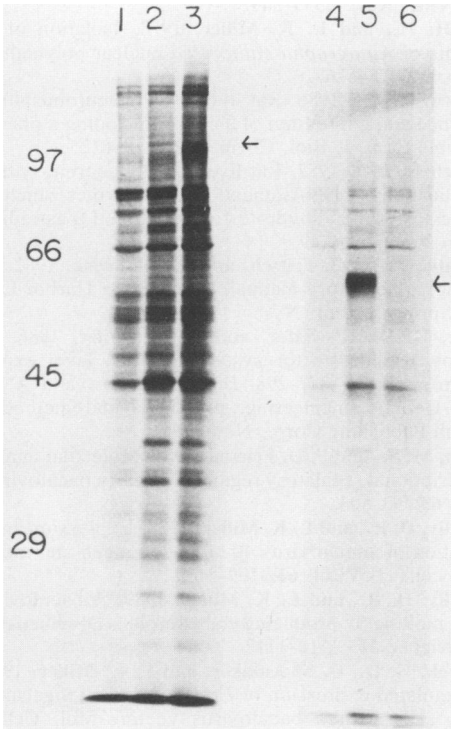

FIG. 6. Identification of the *egt* protein. Mock-infected (lanes 1 and 4), wt AcMNPV-infected (lanes 2 and 5), and vEGTZ-infected (lanes 3 and 6) SF21 cells were pulse-labeled with [^{35}S]methionine from 5 to 6 h p.i. Proteins secreted during the following hour were collected in phosphate-buffered saline. The cells were collected and lysed separately. Intracellular (lanes 1 and 3) and extracellular (lanes 4 to 6) proteins were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. The arrows indicate the *egt-lacZ* fusion protein (lane 3) and the *egt* protein (lane 5). The sizes (10^3) of the molecular weight markers are given on the left.

is typical of the large number of virus isolates which have sustained a deletion of approximately 1 kb in this region following passage through cultured *T. ni* cells (11). DNA sequence analysis of this mutant shows that it is missing a large portion of the *egt* coding sequence, extending from nt 695 to nt 1779 (Fig. 1D). We have found, by sequence analysis and nuclease protection experiments, that precisely the same alteration has occurred in at least four other variants, and it is likely that all of the mutants of Kumar and Miller (11) lacking 1 kb from this region have sustained the same deletion.

DISCUSSION

The AcMNPV *egt* gene encodes a UDPglucosyltransferase which catalyzes the conjugation of glucose with ecdysteroids, insect molting hormones. Expression of this gene allows the virus to prevent the infected insect larva from molting or pupating (19). The demonstration that *egt* functions at this level provides an explanation for the dispensability of *egt* in cell culture. The frequency at which *egt* deletion mutants are found following serial passage of the virus suggests that viral strains lacking this gene may have a selective advantage in tissue culture (11). The reasons for such an advantage are not yet clear.

A remarkable feature of the loss of *egt* upon serial passage in *T. ni* is the specificity of the deletion incurred. Kumar and Miller (11) found that 335 of 667 plaque isolates, derived from 24 independent serial passages, had sustained a similar deletion in this region. We have sequenced the appropriate regions of two of these deletion mutants and determined the mutations to be identical. In addition, we mapped the deletion endpoints of three other mutants (chosen at random) by a nuclease protection assay and again found them to be precisely the same as the mutants sequenced. It is therefore highly likely that all 335 variants altered in this region have sustained precisely the same deletion. We are not sure of the basis of such specificity. The nucleotide sequences of regions flanking the deletions or of the deleted DNA contain no evident structural features such as direct or inverted repeats which might account for such specificity. It is possible that more subtle structural elements are involved.

The transcriptional characterization of the *egt* gene revealed that it is transcribed as two 5′-coterminal mRNAs. These RNAs initiate 43 nt upstream of the *egt* initiation codon. The smaller 1.8-kb transcript, which is the more abundant, encompasses only the *egt* gene, while the larger 3.0-kb RNA also spans the downstream DA26 gene (O'Reilly et al., submitted). These two transcripts are apparently regulated coordinately and behave as early transcripts since their synthesis is not dependent on prior viral transcription or DNA replication. Examination of the DNA sequence upstream of the *egt* start site reveals the presence of a canonical TATA box from 29 to 25 nt before the initiation site (Fig. 1). In addition, the sequence ATTGTGTTA occurs from 47 to 39 nt upstream of the start site. This resembles the sequence motif a/ctcGTGTnc/t previously identified upstream of several baculovirus early genes (17, 24). However, there is as yet no direct evidence for a functional role of either of these elements in the control of early gene expression.

The *egt* protein is the first AcMNPV gene product known to be secreted. Examination of the predicted amino acid sequence reveals that the N terminus is quite hydrophobic and may therefore constitute a signal sequence for export of the protein. *egt* is the second viral protein likely to have an N-terminal signal sequence, the other being the viral envelope protein gp67 (27). Further characterization of such signal sequences may be of use for the design of baculovirus expression vectors which secrete cloned gene products.

The *egt* protein migrates as a diffuse band of around 60 kDa in denaturing polyacrylamide gels (Fig. 6). Indeed, a close examination of the original autoradiograph presented in Fig. 6 revealed the presence of at least two distinct species. Several mammalian UDPglucuronosyltransferases, which are related to *egt* (19), are known to be modified by N-linked glycosylation (e.g., see reference 13). The predicted amino acid sequence of *egt* contains a total of seven potential N-glycosylation sites (Asn-X-Ser/Thr; Fig. 1).

The temporal patterns of accumulation of *egt* transcripts and glucosyltransferase activity are consistent with one another. Thus, RNA levels peak 3 to 6 h p.i., while enzyme activity is maximal from 9 h p.i. onwards. It is interesting that transferase activity can be detected in the extracellular fluid up to 48 h after infection even though *egt*-specific transcripts are virtually undetectable by 24 h p.i. This finding indicates that the *egt* protein is highly stable in the extracellular environment and raises the possibility that, in the insect host, the virus may control molting throughout the infectious cycle without having to continuously synthesize *egt*. The secretion of the *egt* protein contrasts with the homologous

UDPglucuronosyltransferases of mammals, which are membrane bound and thought to function in the lumen of the endoplasmic reticulum (8, 10, 13, 14). The mammalian glucuronosyltransferases possess a highly charged region at the C terminus which is proposed to function as a halt-transfer sequence and anchor the enzyme in the membrane (8, 10, 13, 14). Comparison of the amino acid sequence of *egt* with those of several mammalian transferases reveals that *egt* is approximately 30 amino acids shorter at the C terminus and does not include residues homologous to the halt-transfer sequence (19). It seems likely that baculoviruses acquired the *egt* gene from their insect hosts. It will be interesting to see whether the insect enzyme is secreted or whether the secretion of *egt* is an adaptation unique to the virus.

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LITERATURE CITED

1. **Casadaban, M. J., A. Martinez-Ariaz, S. K. Shapiro, and J. Chow.** 1983. Beta-galactosidase gene fusions for analysing gene expression in *E. coli* and yeast. Methods Enzymol. **100**:293–303.
2. **Crawford, A. M., and L. K. Miller.** 1988. Characterization of an early gene accelerating expression of late genes of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J. Virol. **62**:2773–2781.
3. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology. Elsevier Science Publishing, Inc., New York.
4. **Devereaux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–396.
5. **Dutton, G. F. (ed.).** 1980. Glucuronidation of drugs and other compounds. CRC Press, Boca Raton, Fla.
6. **Guarino, L. A., and M. D. Summers.** 1988. Functional mapping of *Autographa californica* nuclear polyhedrosis virus genes required for late gene expression. J. Virol. **62**:463–471.
7. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**: 351–359.
8. **Jackson, M. R., L. R. McCarthy, D. Harding, S. Wilson, M. W. H. Coughtrie, and B. Burchell.** 1987. Cloning of a human liver microsomal UDP-glucuronosyltransferase cDNA. Biochem. J. **242**:581–588.
9. **Kerkut, G. A., and L. I. Gilbert (ed.).** 1985. Comprehensive insect physiology biochemistry and pharmacology, vol. 7 and 8. Pergamon Press, Oxford.
10. **Kimura, T., and I. S. Owens.** 1987. Mouse UDP-glucuronosyltransferase: cDNA and complete amino acid sequence and regulation. Eur. J. Biochem. **168**:515–521.
11. **Kumar, S., and L. K. Miller.** 1987. Effects of serial passage of *Autographa californica* nuclear polyhedrosis virus in cell culture. Virus Res. **7**:335–349.
12. **Lee, H. H., and L. K. Miller.** 1978. Isolation of genotypic variants of *Autographa californica* nuclear polyhedrosis virus. J. Virol. **27**:754–767.
13. **Mackenzie, P. I.** 1986. Rat liver UDP-glucuronosyltransferase: sequence and expression of a cDNA encoding a phenobarbitol-inducible form. J. Biol. Chem. **261**:6119–6125.
14. **Mackenzie, P. I.** 1987. Rat liver UDP-glucuronosyltransferase: identification of cDNAs encoding the enzymes which glucuronidate testosterone, dihydrotestosterone, and β-estradiol. J. Biol. Chem. **262**:9744–9749.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Miller, D. W., P. Safer, and L. K. Miller.** 1986. An insect baculovirus host-vector system for high level expression of foreign genes, p. 277–298. *In* J. K. Setlow and A. Hollander (ed.), Genetic engineering: principals and methods, vol. 8. Plenum Publishing Corp., New York.
17. **Nissen, M. S., and P. D. Friesen.** 1989. Molecular analysis of the transcriptional regulatory region of an early baculovirus gene. J. Virol. **63**:493–503.
18. **O'Reilly, D. R., and L. K. Miller.** 1988. Expression and complex formation of simian virus 40 large T antigen and mouse p53 in insect cells. J. Virol. **62**:3109–3119.
19. **O'Reilly, D. R., and L. K. Miller.** 1989. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. Science **245**:1110–1112.
20. **Pennock, G. D., C. Shoemaker, and L. K. Miller.** 1984. Strong and regulated expression of *Escherichia coli* β-galactosidase in insect cells with a baculovirus vector. Mol. Cell. Biol. **4**: 399–406.
21. **Pustell, J., and F. C. Kafatos.** 1984. A convenient and adaptable package of computer programs for DNA and protein sequence management, analysis and homology. Nucleic Acids Res. **12**: 643–655.
22. **Sanger, F., S. Nicklen, and A. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Smith, J. N.** 1977. Comparative detoxication of invertebrates, p. 219–232. *In* D. V. Parke and R. L. Smith (ed.), Drug metabolism—from microbe to man. Taylor and Francis, London.
24. **Tomalski, M. D., J. Wu, and L. K. Miller.** 1988. The location, sequence, transcription, and regulation of a baculovirus DNA polymerase gene. Virology **167**:591–600.
25. **Vaughn, J. L., R. H. Goodwin, G. L. Thompkins, and P. McCawley.** 1977. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera:Noctuidae). In Vitro **13**:213–217.
26. **Weaver, R. F., and C. Weissman.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ termini of 15S beta-globin mRNA precursor and mature 10S beta-globin mRNA have identical map coordinates. Nucleic Acids Res. **7**:1175–1193.
27. **Whitford, M., S. Stewart, J. Kuzio, and P. Faulkner.** 1989. Identification and sequence analysis of a gene encoding gp67, an abundant envelope glycoprotein of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J. Virol. **63**:1393–1399.
28. **Yannisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.