Mapping *Pieris rapae* Granulosis Virus Transcripts and Their In Vitro Translation Products

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Poly(A)⁺ RNA was isolated from *Pieris rapae* granulosis virus (PrGV)-infected *P. rapae* larvae at times early (40 h postinoculation) and late (88 h postinoculation) in larval infection. Using Northern (RNA) blot analysis, we determined the sizes, relative abundances, and map locations of over 100 PrGV transcripts. Differences were found in the transcripts which had accumulated at the two time points. Splicing of these transcripts was not detected. Evidence for the expression of overlapping RNAs in PrGV was obtained. A minimum of 35 PrGV translation products were detected via hybrid selection of poly(A)⁺ RNA with specific PrGV restriction endonuclease fragments, followed by in vitro translation.

Baculoviruses are studied because of their potential as biological pesticides (6, 29, 30) and, more recently, because of their use as vectors for the expression of foreign genes in a eucaryotic system (16, 25, 26). The 128-kilobase-pair (kbp) circular DNA genome of a subgroup A baculovirus, *Autographa californica* nuclear polyhedrosis virus (AcMNPV), has been characterized by physical mapping (5, 28, 33), DNA sequencing of specific areas (16, 18, 39), transcriptional studies (10, 12, 21-23, 31, 35), and in vitro translation of hybrid-selected viral transcripts (11, 27, 34).

These studies have discovered no evidence for the splicing of viral transcripts; rather, the studies reveal the expression of AcMNPV genes by temporally regulated sets of overlapping mRNAs which may have either a common 5' or a common 3' terminus. Of the many in vitro translation products found to date, only two have been functionally identified: (i) the 28-kilodalton (kDa) polyhedrin protein, which is the major structural component of the paracrystalline occlusion body of the virus (35), and (ii) the 6.9-kDa core protein of viral nucleocapsids (39). The function of another AcMNPV gene, the immediate-early IE-1 gene, was determined via a functional assay (15) to be a *trans*-acting regulatory gene responsible for expression of the delayed-early gene coding for the major 39-kDa nonstructural protein.

Less information is available on the gene expression of subgroup B baculoviruses, the granulosis viruses (GV), because of the difficulty of their in vitro replication in tissue culture. However, the physical maps of two GVs, *Cydia pomonella* GV (8) and *Pieris rapae* GV (PrGV; 9), have been established, along with the locations of the granulin genes on these genomes. The granulin genes of *Trichoplusia ni* GV (1) and *Pieris brassicae* GV (PbGV) (4) have been sequenced.

In this report, we present transcriptional studies of the 106-kbp circular DNA of PrGV with infected larvae as sources of viral mRNAs. By Northern (RNA) blot analysis, over 100 viral transcripts isolated early and late during larval infection were mapped on the PrGV genome. No evidence for the splicing of these transcripts was found. Expression of PrGV genes by the transcription of overlapping sets of RNAs was suggested. A translational map of 36 viral polypeptides was established by in vitro translation of hybrid-selected transcripts.

MATERIALS AND METHODS

Rearing of *P. rapae* larvae and PrGV inoculation. PrGV was obtained from Robert Jacques (Research Station for Agriculture, Harrow, Ontario, Canada). *P. rapae* larvae were reared in 16-oz (ca. 473-ml) Styrofoam cups on high wheat germ diet at 28°C (3). Larvae at late instar 4 were inoculated with PrGV by surface contamination of the diet with 1.5×10^7 capsules per cm².

Electron microscopy study of viral replication in PrGV-infected larvae. Midgut, tracheoblasts, fat body, muscle, and epidermal tissue were dissected out of PrGV-infected *P. rapae* larvae 12, 18, 24, 36, 48, 72, 84, and 96 h postinoculation (p.i.) and analyzed by transmission electron microscopy for the progress of viral replication, as described by Granados and Lawler (14).

Preparation of PrGV DNA and viral restriction fragment DNA. PrGV DNA was isolated as reported by Dwyer and Granados (9). A library of cloned PrGV *Eco*RI fragments was made and propagated by the methods of Maniatis et al. (24). Clones were not obtained for PrGV *Eco*RI fragments A, E, and K or for PrGV *Kpn*I fragment N. These fragments were obtained from gel bands by the methods of Maniatis et al. (24) after digestion of PrGV DNA with restriction enzymes *Eco*RI and *Kpn*I according to directions of the manufacturer (Bethesda Research Laboratories, Inc.).

Poly(A)⁺ RNA isolation from PrGV-infected *P. rapae* larvae. At 40 and 88 h p.i., PrGV-infected larvae were combined with 5 volumes by weight of solution A (5 M guanidinium isothiocyanate, 20 mM Tris hydrochloride [pH 7.5], 25 mM EDTA, 0.1 M β -mercaptoethanol, 0.5% Sarkosyl [CIBA-GEIGY Corp.]) and homogenized in a Waring blender for 1 min on high speed. The homogenate was filtered through four layers of cheesecloth and centrifuged for 5 min at $500 \times g$ in a GLC tabletop centrifuge, and the supernatant was combined with 0.5 volume of ethanol at -20°C overnight to precipitate the nucleic acids. The precipitate was pelleted in a Sorvall RC5 centrifuge at $12,000 \times g$ for 20 min. The pellet was suspended in solution A, combined with an equal volume of solution B (5.7 M CsCl, 10 mM Tris hydrochloride [pH 7.5], 10 mM EDTA), and layered onto 1-ml cushions of solution B in SWTi60 centrifuge tubes. The tubes were centrifuged in the SWTi60 rotor for 16 h at $165,000 \times g$ at 20°C to pellet the RNA. RNA pellets were suspended in sterile distilled H₂O with 10 mM vanadyl ribonucleoside complex and put through oligo(dT)

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columns by the method of Aviv and Leder (2). Poly(A)⁺ RNA was precipitated with 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol and stored at -100°C. Vanadyl ribonucleoside complex and oligo(dT) were obtained from Bethesda Research Laboratories.

Northern blot preparation and hybridization. To prepare Northern blots, 5 µg of poly(A)⁺ RNA per well was separated by electrophoresis in denaturing 1.5% agarose gels containing 2.2 M formaldehyde and blotted onto nitrocellulose filters as described by Maniatis et al. (24). Cucumber mosaic virus RNA, obtained from Dennis Gonsalves (New York State Experimental Station, Geneva, N.Y.), was used for molecular weight markers on these gels. Probes were labeled with [α -³²P]dTTP by nick translation (32) to specific activities of between 10⁷ and 10⁸ cpm/µg. Probed blots were pretreated at 42°C overnight in 60% formamide-0.9 M NaCl-50 mM sodium phosphate (pH 7.0)-5 mM EDTA-0.1% sodium dodecyl sulfate (SDS)-0.1% Denhardt solution (0.2% Ficoll [Pharmacia Fine Chemicals], 0.2% polyvinylpyrrolidone, 0.02% bovine serum albumin)-200-µg/ml denatured salmon sperm DNA and hybridized with denatured probe in the same solution but containing 0.02% Denhardt solution. The blots were washed as follows: (i) 20 min twice in 0.36 M NaCl-20 mM sodium phosphate (pH 7.0)-2 mM EDTA-0.1% SDS at room temperature, (ii) 20 min twice in 1× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate, pH 7.0)-0.1% SDS at 37°C, and (iii) 20 min twice in 0.1× SSC-0.1% SDS at 60°C. The filters were air dried and autoradiographed by exposure to XRP1 film (Eastman Kodak Co.) with an intensifying screen (Du Pont Co.) at -80°C.

Hybrid selection and in vitro translation of PrGV transcripts. PrGV DNA and specific PrGV restriction fragment DNAs derived from either clones or gel bands (*Eco*RI fragment E, *Bam*HI fragment I, and *Kpn*I fragment N) were applied to diazobenzoyloxymethyl or nitrocellulose filters, or both, and used for the hybrid selection of PrGV transcripts as described by Maniatis et al. (24). In vitro translations were carried out in the Rabbit Reticulocyte Lysate (nuclease treated) of Promega as suggested by the manufacturer. In vitro translation polypeptides labeled with either [³⁵S]methionine or [³H]leucine were separated on 12.5% (wt/vol) SDS-polyacrylamide gels (19), treated with En³Hance (New England Nuclear Corp.), and autoradiographed. ¹⁴C-labeled proteins (New England Nuclear) were used as molecular weight markers. XRP1 film (Eastman Kodak) was preflashed (20) before autoradiography with ³H-labeled polypeptides.

Immunoprecipitation of in vitro translation polypeptides. PrGV occluded virus (OV) antiserum was raised in rabbits in this laboratory as described by Volkman (38). PbGV OV antiserum was obtained from Norman Crook (7). The reactions of these antisera against PrGV OV and granulin proteins were characterized by Western blot (immunoblot) analysis as described by Volkman (38). Immunoprecipitations were done as described by Vlak et al. (37) with the indicated polyclonal antiserum.

Two-dimensional nuclease S1 gel mapping. Analysis for the splicing of PrGV transcripts by the two-dimensional nuclease S1 method was done as described by Lübbert and Doerfler (21). Analysis of PrGV transcripts in the area of *Kpn*I fragment N by two-dimensional nuclease S1 gel mapping was done as described by Lübbert and Doerfler (22).

RESULTS

Progress of viral replication in *P. rapae* larvae. Progeny virions were first observed by electron microscopy in fat

body tissue 36 h p.i. (data not shown). Thereafter, virus and capsule concentrations increased in tracheoblasts, fat body, muscle, and epidermis. No virus was observed in midgut tissue at any of the time points. Infected *P. rapae* larvae died shortly after 96 h p.i.

Characterization of poly(A)⁺ RNA from infected larvae. Poly(A)⁺ RNAs isolated from healthy and PrGV-infected *P. rapae* larvae 40 and 88 h p.i. were translated in vitro, and the polypeptides produced were analyzed on an SDS-polyacrylamide gel (Fig. 1A). At 40 h p.i., a 42-kDa polypeptide that was not seen in healthy larvae or not seen as clearly in the sample at 88 h p.i. was abundant. Also at 40 h p.i., some polypeptides produced in healthy larvae were no longer visible in infected larvae, especially a 21-kDa polypeptide. At 88 h p.i., many polypeptide bands were detected which were not seen or were less abundant in the healthy larvae or the samples at 40 h p.i. Some of these polypeptides were viral structural proteins. We have identified granulin, the major capsid protein, and 13 other viral structural proteins by immunoprecipitation of the in vitro translation products isolated at 88 h p.i. (Fig. 1B) with polyclonal antisera raised against PrGV OV and PbGV OV. During characterization of these antisera against PrGV OV and granulin by Western

FIG. 1. Autoradiographs of [³H]leucine-labeled in vitro translation polypeptides from the indicated poly(A)⁺ RNA preparations separated on 12.5% SDS-polyacrylamide gels. (A) Polypeptides resulting from in vitro translation of poly(A)⁺ RNA isolated from healthy larvae and PrGV-infected larvae at 40 and 88 h p.i. Arrowheads highlight the 40-h-p.i. 42-kDa (K) polypeptide and the 88-h-p.i. 34-kDa major capsid and the 30-kDa granulin polypeptides identified as such by immunoprecipitation. (B) Polypeptides resulting from in vitro translation of poly(A)⁺ RNA isolated from PrGV-infected larvae at 88 h p.i. and immunoprecipitated with antiserum raised against PbGV OV or PrGV OV. (C) Polypeptides resulting from in vitro translation of combined poly(A)⁺ RNA isolated from PrGV-infected larvae at 40 and 88 h p.i. and hybrid selected with PrGV DNA. Molecular mass standards of ¹⁴C-labeled proteins are at the left of each panel.

blot analysis, we found that the PrGV OV antiserum reacted only with PrGV major capsid protein (34 kDa) and granulin (30 kDa), whereas the PbGV OV antiserum reacted with 20 PrGV OV structural proteins, including the major capsid protein but excluding the granulin protein (data not shown). The molecular masses of these in vitro translation viral structural polypeptides (Fig. 1B) were 50, 48, 34 (major capsid protein), 30 (granulin), 29, 26, 25, 24, 22, 19, 18, 17, 15, and 14 kDa. At least 18 viral polypeptides (Fig. 1C) were produced by in vitro translation of combined poly(A)⁺ RNA isolated from *P. rapae* larvae infected with PrGV for 40 and 88 h and hybrid selected with total PrGV DNA. The molecular masses of these virus-specific in vitro translation products were 74, 54, 52, 45, 41, 38, 37, 35, 34, 32, 30, 26, 23, 22, 19, 18, 17, and 15 kDa. These results reflect the ability of PrGV-infected *P. rapae* larvae to provide viral RNA for translational studies.

PrGV transcripts and their in vitro translation products. Poly(A)⁺ RNA was isolated from PrGV-infected larvae at 40 and 88 h p.i. and used for Northern blot analysis. Autoradiograms of these Northern blots in the order of the positions of their probes along the PrGV genome are presented in Fig. 2. Below each set of Northern blots is an *EcoRI* restriction map of the PrGV genome. The circular PrGV genome is linearized in *EcoRI* fragment F, before *KpnI* fragment N (0.0 to 0.6 map units), which is the smallest restriction fragment containing the granulin gene (9). Below the physical map are reported schematically the molecular masses of the in vitro translation polypeptides detected after hybrid selection of poly(A)⁺ RNA isolated at 40 or 88 h p.i. by the *EcoRI* fragments indicated. A sample of the hybrid-selected in vitro translation results is shown in Fig. 3.

Over 100 size classes of viral RNAs were detected by Northern blot analysis of poly(A)⁺ RNA isolated at 40 and 88 h p.i. from the PrGV-infected larvae. It was interesting to see many differences in the sizes and abundances of viral RNAs found at 40 and 88 h p.i. Major transcripts which were relatively more abundant early (40 h p.i.) than late (88 h p.i.) in larval infection mapped to *EcoRI* fragments F (2.4 kilobases [kb]), M (0.6 kb), C (1.1, 0.6, and 0.45 kb), E (0.8 kb), B (0.8 and 0.5 kb), and P (0.6 kb). These regions are distributed throughout the PrGV genome. A 42-kDa in vitro translation polypeptide, corresponding in size to the major 42-kDa polypeptide seen after in vitro translation of total poly(A)⁺ RNA isolated at 40 h p.i. (Fig. 1A), was encoded by a 40-h-p.i. viral transcript(s) hybrid selected by a clone of *EcoRI* fragment C at PrGV genome map positions 31 to 39 (Fig. 3). Indeed, by using PrGV *KpnI* fragment L (map units 35 to 36) and PrGV *BamHI* fragment I (map units 36 to 38.5) as probes of Northern blots and for hybrid selection of 40-h-p.i. poly(A)⁺ RNA, we have further located the major early 1.1- and 0.6-kb transcripts and the 42-kDa in vitro translation product to *BamHI* fragment I map positioned at 36 to 39 map units (data not shown).

Hybrid selection of early (40-h-p.i.) poly(A)⁺ RNA with the PrGV *EcoRI* fragment B clone followed by in vitro translation resulted in unique polypeptides of 40, 39, 38, 33, 26, and 20 kDa. The 33- and 20-kDa polypeptides were not detected in the hybrid selection of late (88-h-p.i.) poly(A)⁺ RNA by this region, though the 39-kDa polypeptide was relatively more abundant at this time. Hybrid selection of early (40-h-p.i.) poly(A)⁺ RNA with PrGV *EcoRI* fragments M and P followed by in vitro translation resulted in a 17-kDa polypeptide mapping to the M region and a major 37-kDa and minor 32- and 31-kDa polypeptides associated with the P region.

Late in larval infection, viral poly(A)⁺ RNA transcribed from every *EcoRI* fragment of the PrGV genome had accumulated in infected larvae. Some of the most abundant late transcripts were in the regions of *EcoRI* fragment F-*KpnI* fragment N, *EcoRI* fragment D, *EcoRI* fragment O, *EcoRI* fragment Q, and *EcoRI* fragment E. The in vitro translation products for these regions and others are shown in Fig. 2. We have previously reported (9) the location of the granulin gene in PrGV by cross-homology with a clone containing *T. ni* GV granulin gene sequences (1) to PrGV 0.6-kbp *KpnI* fragment N at 0.0 to 0.6 map units in *EcoRI* fragment F (94 to 0.6 map units). Northern blot analysis of the 0.6-kbp *KpnI* fragment N region revealed two major (2.1 and 1.0 kb) and many minor (3.6, 3.0, 2.5, 1.7, 1.5, 1.25, and 1.15 kb) size classes of viral RNAs transcribed from this area. Hybrid selection with *KpnI* fragment N followed by in vitro translation yielded a 30-kDa polypeptide which comigrated with PrGV granulin protein (data not shown). The functions of other in vitro translation polypeptides have yet to be determined.

Since there were so many size classes of viral poly(A)⁺ RNA, as revealed by Northern blot analysis, it is difficult to judge where these size classes of viral RNAs are transcribed across *EcoRI* restriction sites and, thus, where they are detected by adjacent *EcoRI* fragments. However, *EcoRI* fragments O and Q seem to be possible areas where the 1.85-, 1.5-, 0.85-, and/or 0.75-kb RNA may be shared by the two regions of the genome. Indeed, hybrid selection and in vitro translation with clones of these two fragments yielded comigrating polypeptides of 33 and 16 kDa from both fragments (Fig. 3). Whether these polypeptides are identical has yet to be determined.

Splicing of PrGV transcripts. Total viral DNA was hybridized with combined poly(A)⁺ RNA isolated from larvae at 40 and 88 h p.i. and then treated with S1 nuclease to digest all single-stranded nucleic acids. The DNA-RNA hybrids were separated on a two-dimensional 1.2% agarose gel first under neutral conditions and then by electrophoresis under denaturing alkaline conditions. The gel was blotted onto a nitrocellulose filter and then probed with total PrGV DNA sequences (Fig. 4A). If any of the viral RNAs are spliced, one would detect DNA below the diagonal resulting from the separation of transcript DNA at the splice junctions after the degradation of transcript RNA by the alkaline conditions of the second dimension. No PrGV DNA was detected below the diagonal.

Two-dimensional nuclease S1 mapping of PrGV in the granulin gene region. Viral DNA digested with restriction enzyme *EcoRI* was hybridized with total poly(A)⁺ RNA isolated from larvae at 88 h p.i. and then treated with S1 nuclease. The DNA-RNA hybrids were separated on a two-dimensional 1.5% agarose gel as described above. After DNA was blotted onto nitrocellulose, the DNA was probed with nick-translated PrGV *KpnI* fragment N sequences (Fig. 4B). No PrGV DNA was detected below the diagonal. Three bands, the major 0.75-kb and two minor 1.0- and 2.0-kb bands, were detected on the diagonal.

DISCUSSION

In this work, the sizes, relative abundances, and locations on the PrGV genome of over 100 viral transcripts were determined. Differences in viral transcripts accumulated early (40 h p.i.) and late (88 h p.i.) in larval infection were found. Electron microscopy studies of AcMNPV-infected *T. ni* larvae suggested a progression of viral growth where

FIG. 2. Autoradiograms of Northern blot analyses of PrGV-specific poly(A)⁺ RNAs isolated from larvae 40 (E) and 88 (L) h p.i. Except for *EcoRI* fragments A, E, and KL (comigrating) and *KpnI* fragment N, which were isolated from gel bands, cloned *EcoRI* fragments were used as probes, and blots are presented in the genomic order of these PrGV *EcoRI* (and *KpnI*) fragments starting at the end of *EcoRI* fragment F. Molecular masses of the RNA classes (indicated at the sides of the gels) are the averages of at least two determinations. In some cases, the transcripts of the late time point were overexposed so that the transcripts of the early time point can be seen (see PrGV transcripts of *EcoRI*

fragments F, D, O, Q, and E and *KpnI* fragment N). In these cases, a lighter exposure of the late sample is presented on the right side of the autoradiogram. Below each group of blots is a schematic representation of the *EcoRI* physical map for the PrGV genome (9) with map units reported above and the locations and molecular masses of hybrid-selected in vitro translation products reported below. The in vitro translation data were also the results of at least two determinations. Molecular masses in parentheses indicate in vitro translation products which were more abundant in poly(A)⁺ RNA isolated at 40 h p.i. K, Kilodalton. The horizontal lines at the sides of the gels indicate the migration positions of the molecular mass markers.

FIG. 3. Autoradiogram of [^{35}S]methionine-labeled in vitro translation polypeptides from poly(A) $^{+}$ RNA isolated at either 40 or 88 h p.i. PrGV-infected larvae were hybrid selected with PrGV *EcoRI* fragment D, O, F, and Q clones and separated on 12.5% SDS-polyacrylamide gels. The result of a blank (no RNA added) in vitro translation is included. The arrowheads indicate hybrid-selected in vitro translation products. K, Kilodalton. Molecular mass standards of ^{14}C -labeled proteins are indicated at the left.

progeny virus is seen in midgut tissue first and then continues the systemic infection (13). In our study, electron microscopy examination of PrGV-infected *P. rapae* larvae showed progeny virus in several internal tissues initially at 36 h p.i. No progeny virus was seen in midgut cells even late in infection. Therefore, PrGV did not demonstrate the progression of viral growth seen in AcMNPV-infected larvae.

Since viral infection of larval tissues is asynchronous, we cannot conclude that the progression of transcripts and their proteins that we have detected during the course of larval infection reflects the true temporal expression of viral genes. Indeed, electron microscopy analysis detected progeny virus 36 h p.i. in the fat body tissue, hours before the early time point. We are really determining what viral transcripts have accumulated at these times in infected larvae as a result of the degree of infection in various cells, the time and level of transcription, and the stability of the transcripts.

Using the method of Lübbert and Doerfler (21), we found no evidence for the splicing of PrGV transcripts. Of course, this method does not rule out splicing in a minority population of viral RNAs or splices close to the ends of viral transcripts. These results are similar to those found for AcMNPV (21) and support the hypothesis that the lack of major splicing of viral transcripts is a general baculovirus phenomenon.

Inspection of some of the Northern blots (for example, the 2.3-, 1.8-, and 1.5-kbp *EcoRI* fragments O, P, and Q, respectively, and the 0.6-kbp *KpnI* fragment N) revealed a number and size of transcripts which exceed the coding capacity of the single-strand equivalent of that genomic region, even when the crossing over of some of these transcripts into adjacent *EcoRI* fragment sequences is considered. Since we have not detected major splices, the transcription of sets of overlapping RNAs, as was found to be the case for AcMNPV gene expression, seems the best explanation for the excessive amounts of RNA. Using two-dimensional nuclease S1 mapping, we studied the transcripts in the area of the 0.6-kbp *KpnI* fragment N (0.0 to 0.6 map unit) more closely. As suggested in our previous report (9), the granulin gene coding sequences may be at 99.9 to 0.6 map units, which includes the entire *KpnI* fragment N sequence. *KpnI* fragment N abuts the edge of *EcoRI* fragment F. The sequences of the 2.0-, 1.0-, and 0.75-kb S1 nuclease digestion products detected must be located in *KpnI* fragment N. Since none of these bands yielded smaller DNA fragments in the second dimension, a result reflective of trimolecular hybridization among the RNAs and *EcoRI* fragments F and D, we think these bands do not cross into

FIG. 4. Autoradiograms of two-dimensional S1 nuclease mapping of DNA-RNA hybrids on 1.2% agarose gels. (A) Poly(A) $^{+}$ RNA isolated from PrGV-infected larvae at 40 and 88 h p.i., hybridized with PrGV DNA, S1 nuclease digested, separated on the gel in a neutral pH (N) followed by an alkaline pH (A), and probed with PrGV [^{32}P]DNA. (B) Poly(A) $^{+}$ RNA isolated from PrGV-infected larvae at 88 h p.i., hybridized with *EcoRI*-digested PrGV DNA, S1 nuclease digested, separated on the two-dimensional gel, and probed with ^{32}P -labeled PrGV *KpnI* fragment N. Molecular mass markers were 1-kbp ladder DNA purchased from Bethesda Research Laboratories.

*Eco*RI fragment D, but must extend from *Kpn*I fragment N further into *Eco*RI fragment F. Therefore, some of their sequences must overlap. The major 750-bp band may correspond to the major granulin transcript of 1.0 kb seen in the Northern blots after addition of the poly(A) tail.

By using hybrid selection, we mapped many in vitro translation products along the PrGV genome, but the function of only one, the granulin protein mapping to *Kpn*I fragment N at 0.0 to 0.6 map units, is known. In the future, it should be possible to identify some of these in vitro translation polypeptides as at least viral structural proteins by hybrid selection and in vitro translation followed by immunoprecipitation with antisera against PrGV OV and PbGV OV. We suspect that areas of the PrGV genome, such as *Eco*RI fragments D, O, Q, and E, from which abundant transcripts have accumulated late in larval infection, are good candidates for such analysis.

Another approach to determining the function of PrGV proteins may be by comparison with other better characterized baculoviruses. We did this by using *T. ni* GV granulin gene sequences to identify the PrGV granulin gene (9). We have reported (9) a similar analysis to search for the PrGV gene and protein of similar function as the major early (39-kDa) and late (10-kDa) nonstructural proteins of AcMNPV. Under low-stringency conditions, we found no cross-homology between the AcMNPV sequences of these proteins and PrGV sequences. More recently, we have searched for the PrGV sequences coding for the small arginine-rich core polypeptide by using cDNA clone pHD2 with sequences for the equivalent protein of AcMNPV (39). Again, we found no cross-homology between the AcMNPV probe and PrGV sequences (data not shown). PrGV has a 16.5-kDa core protein (36; unpublished results), but its sequences must have diverged more than 40% in comparison with the AcMNPV cDNA sequences to be undetected by the hybridization conditions used in this analysis. As previously reported (9), we have found other areas of cross-homology between the PrGV and AcMNPV genomes. These areas might contain genes of similar function between the two baculoviruses. As the functions of the AcMNPV genes are determined, the functions of the corresponding PrGV genes may be elucidated.

Of course, a breakthrough in the in vitro replication of PrGV in tissue culture would provide, via genetic manipulation of viral sequences, the best means for ascribing functions for PrGV gene products. Several new cell lines, derived from *P. rapae* embryonic tissue, have been established in our laboratory, but none of these lines are susceptible to PrGV (manuscript in preparation).

ACKNOWLEDGMENTS

We thank Norman Crook for the PbGV OV antiserum. The cDNA clone pHD2 was the generous gift of Lois Miller.

This work was supported by a grant from the Cornell Biotechnology Program, which is supported by the New York Science and Technology Foundation and a consortium of industries.

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