PN-ABM-554

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 11/9/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C7-150
PR #2

Attachment

PN-ABM-554
ISN 79659
C7-150

DENSOVIRUSES AS INSECTICIDES

GRANT NO. 936-5544-G-00-7016-00

PROGRESS REPORT NO. 2 - ISRAELI GROUP

25 October 1988

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Abstract

A 5.4 kb DNA fragment, representing the entire coding region of GmDNV was cloned, and about 2500 bases (from non-contiguous regions) were so far sequenced. The available sequences revealed some properties which are both common and unique amongst parvoviruses. The sequence is extremely AT rich. Sequences at the 3' and 5' termini contain a 250 base inverted repeat, not found in parvoviruses yet. There is no homology between GmDNV DNA and that of other known parvoviruses, including *Bombyx mori* DNV DNA. In general the basic structural features of the viral DNA, such as the packaging of both viral strands in separate virions, and the identical (repeated) sequences on both termini, bear structural similarity the human parvoviruses adeno-associated virus (AAV) and B19.

In the absence of a tissue culture host for any of the densoviruses, expression studies were conducted in larvae infected or injected with the virus. Using hybridization "riboprobes" of known orientations, we found that all viral RNA was transcribed from one DNA strand only. Northern analysis identified 4 abundant, poly (A)-containing transcripts in infected larvae, but also in one insect cell line.

GmDNV isolated from larvae yielded 3 density classes of 1.32, 1.40 and 1.42 g/cm^3. The 1.32 band is composed of "empty" virions devoid of DNA. The other two bands contain a uniform species of viral DNA of approximately 6 kb), but the DNA/protein ratios differ between them. All 3 bands yield 4 polypeptides (45K, 55K, 65K and 92K) at varying ratios. *In vitro* translation of poly (A)-containing RNA yielded a prominent 45K polypeptide, indicating that at least this capsid protein is not modified post-translationally.

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Materials and Methods.

Larvae, cells, virus and viral plasmids. Larvae of Galleria mellonella were grown on a food mixture containing Gerber's baby food (Mixed Cereal), honey and glycerol. Larvae were propagated in polypropylen boxes. For venting purposes, a stainless steel screen was welded into the box covers. The boxes were incubated at a constant 30°C temperature, with 50% humidity. The pupae were transferred to larger boxes without the addition of fresh food, and allowed to lay eggs on a multi-folded wax paper. To obtain synchronous larval cultures, eggs laid during a 24-48 h period were transferred to each box. To obtain an "all-year-round" culture, hatching times were controlled by incubating eggs at 4°C and seeding them in food boxes at pre-determined time intervals. Insect cell cultures were obtained from Dr. Dwight Lynn, from USDA in Beltsville, Maryland. Insect cells were grown at 30°C in Grace medium (GIBCO) supplemented with 10% fetal calf serum, and 0.3% each of lactalbumin and yeastolate (Difco). GmDNV was a gift from Dr. Peter Tijssen from Institut Armand Frappier, Montreal, Canada. The virus was grown in fourth instar larvae which were either injected or infected. Dead larvae were collected, ground, filtered through glass wool, and virus particles were purified according to published procedures. To construct a genetic clone, double-stranded viral DNA (see below) was cleaved with BamH1, and the resulting 5.4 kb fragment was ligated into the BamH1 site of pUC19.

Viral DNA and RNA. To obtain single stranded (ss) viral DNA, CsCl-purified virus particles were extracted with phenol-chloroform. Total RNA from infected larvae was prepared by grinding he larvae in 4M guanidine thiocyanate in a Polytron, filtering the extract through glass wool and subjecting the extract to isopycnic centrifugation in CsCl (1.4 g/cc, 12h at 30,000 rpm at 20°C). The RNA

sediments at the bottom and was usually washed once with guanidine hypochloride, then phenol extracted. Poly (A) - containing RNA was selected by chromatographing the RNA through an oligo (dT)-celluiose column. Viral double-stranded (ds) DNA was obtained by two ways. It appeared as a distinct band in CsCl gradients during RNA preparation, and was extracted, and further purified by agarose gel electrophoresis. Alternatively, CsCl-purified virus was extracted at high ionic strength, yielding duplex viral DNA (see below). Size analysis of viral RNA and proteins. RNA was analyzed by the Northern blotting procedure. The probe for hybridization was the the cloned viral DNA, ^{32}P-labeled by nick-translation. Viral proteins were analysed by disrupting purified virus in a buffer containing 2% SDS, followed by SDS-PAGE electrophoresis.

In vitro translation. Poly (A) containing RNA (40 ng) was translated in a rabbit reticulocyte lysate system in the presence of ^{35}S-methionine.

Results

Cloning and sequencing of the GmDNV genome. In the virion, GmDNV DNA (like all other parvoviruses) exists in a single-stranded form, which is converted to a duplex "replicating form" (RF DNA) during productive infection. RF DNA was isolated by two procedures. The first procedure was a simple phenol extraction of CsCl-purified virus preparation, In the presence of 0.1M NaCl. As reported below, the two strands of GmDNV DNA (viral and complementary, or + and - , respectively) are packaged into separate virus particles in an equimolar ratio. Phenol extraction readily yielded ds DNA, about 6 kb in size (see below). In the second procedure, viral RF DNA was obtained from injected larvae, as a side product of RNA preparation by the guanidine method at the CsCl step. Using this

procedure, viral DNA was obtained in ample quantities, but had to be further purified by agarose gel electrophoresis.

BamH1 cleavage of viral RF DNA conveniently yielded a 5.4 kb fragment which, as is shown below, contained the entire coding region of the virus. The BamH1-flanked fragment was ligated into pUC19, and a partial restriction map was constructed using, primarily, enzymes with sites represented in the polylinker of pUC19. Based on the restriction map, subclones representing the entire sequence were subsequently constructed in M13. Using the dideoxy method and "Sequenase" (USB), about 2500 bases (from non-contiguous regions) were sequenced to date. In Fig. 1, the GmDNV clone and the physically-determined restriction sites are shown. Below, are five regions I-V, designating the regions whose sequence is complete. The sequencing strategy is shown by arrows further below. Fig. 2 shows the sequence, with the Roman numerals corresponding to the regions in Fig. 1. All the polylinker sequences were removed, leaving GmDNV sequences only. It should be noted, however, that this sequencing strategy was chosen primarily in order to obtain data about the termini. In parallel, we have created in the 5.4 kbp clone, a series of deletions by Exo III, and these clones, which contain multiple overlaps, are currently being sequenced.

As mentioned above, the fragments at the termini of the viral DNA were given priority in sequencing. This was for two purposes. First, to find out whether this clone contained the entire coding region of GmDNV. Parvoviruses are characterized by terminal repeats on both ends. These repeats contain signals for DNA replication and packaging, but not coding sequences. Therefore, ternminal repeats would indicate that the entire coding sequences are present in the clone. Indeed, computer analysis of the regions I and V showed a perfect inverted terminal repeat of at least 250 nucleotides. The repeat may be even more extensive, since region I was only sequenced to this point, but even so, it is clear that the clone

domains are the terminal repeats, tentatively suggesting (based, of course, on our knowledge from mammalian parvoviruses), that the coding sequences of the virus are entirely contained in the sequence.

The second purpose for the priority in sequencing the termini was a structural consideration. Surprisingly, the terminal repeat found, places GmDNV in that category of parvoviruses with identical termini (i.e., the human AAV and B19), as opposed to those containing, on both ends, palindromic structures, but containing unrelated sequences. There is, however, a major structural difference between that found in AAV and GmDNV. AAV DNA contains a direct repeat of 145 bases, and within it is a palindromic sequence of 125 residues that form a hairpin. The rest of the repeated sequence is short (20 residues), not involved in the hairpin structure, and called "inboard" sequence. In GnDNV, we are already finding an exceptionally long inverted inboard repeat. Hence, this is a unique structure within the parvoviruses, yet the general structural feature of terminal repeats, is maintained. Another unusual feature is the very high percentage of A and T stretches of 6 residues and over. Some regions in the sequence, such as the one marked by an arrow, contains 102 A and T residues out of 125 bases (81% AT).

Computer analysis of the available sequence included homology searches against known sequences of mammalian parvoviruses, and also against that of Bombyx mori DNV (BmDNV), the only invertebrate parvovirus sequenced to date. Such searches revealed a complete lack of homology. Although this data is based on 50% of the sequence only, the lack of homology to BmDNV is surprising, since in mammalian parvoviruses, an extensive region of homology is found,

Structure of GmDNV DNA. There has been one earlier report that extraction of DNA from virus particles in the presence of high salt resulted in ds DNA, while extraction in low salt yielded ss DNA. We repeated this finding using gel electrophoresis and blotting techniques, which were not available at that time.

Indeed we found that DNA extracted in the presence of 0.1 M NaCl migrated in neutral agarose gel as a 6 kbp, while DNA extracted in the absence of NaCl migrated as a 6 kb fragment. Thus, GmDNV seems to package viral and complementary DNA strands in separate virions in a fairly close molar ratio. The only other parvoviruses known to do that are the human AAV and B19, and none of the ones in between in the evolutionary scale.

To study the structure of the termini, which are not included in the 5.4 kb fragment, the following experiments were performed. Viral DNA (extracted at high salt) was cleaved with BamH1, the mixture was heated (10 minutes at 95°C), quick-cooled in ice water, and electrophoresed in 2% agarose alongside with the untreated DNA. The products were then blotted onto nitrocellulose filter and hybridized with ^{32}P-labeled terminal Bam fragment. Untreated samples yielded a 300 bp band while the treated sample migrated as a 150 bp fragment. These experiments indicate that the terminal structures are palindromic and thus bear remarkable structural resemblance to those of the human parvoviruses AAV and B19. This structure is being further pursued.

Viral gene expression in larvae and in tissue culture. A major obstacle in the study of densovirus molecular biology is the absence of a tissue culture system. Our studies are therefore *in vivo* ones, primarily. The larvae (3rd instar on) are either infected or injected with a measured quantity of CsCl-purified virus. Viral RNA is isolated by homogenizing the larvae in 4M guanidine-tiocyanate, followed by centrifugation through CsCl. To determine the orientation of transcription, fragments from 3 regions of the genome were cloned into the ribosystem pGEM-2 (Promega) and radiolabeled "riboprobes", synthesized in both orientations. were used to probe RNA dot blots. The results of two of these regions are shown in Fig. 3a and Fig. 3b, and suggest that, as in mammalian parvoviruses. the viral strand is exclusively transcribed. These results are being expanded now by running RNA

gels, which would also provide us with some idea on the transcriptional organization of the GmDNV genome.

We applied the Northern blotting technique to find out if we can identify discrete transcripts. To do that, poly (A)-containing RNA from injected larvae was selected by chromatography through oligo-dT-cellulose columns and analyzed in formamide gels, followed by hybridization to a radiolabeled probe. Using this procedure, we identified 4 abundant viral, poly $(A)^+$ transcripts (Fig. 4). We have also examined the ability of several insect cell lines (all of which non-permissive to GmDNV) to support viral transcription following GmDNV infection. Viral transcripts (albeit at low levels) were found in one cell line, (TN-368 from Trichoplusia ni ovaries). Furthermore, Northern analysis shows that they are identical in size distribution to the transcripts obtained from larvae, indicating that, despite their non-permissiveness, these cells carry out a faithful transcription of the viral genome, and suggesting that at least earlier events in the viral replication cycle may now be studied *in vitro* Transcription studies in this cell line are underway.

Replication of GmDNV as a function of larval development. Larvae from instars 3-5, and from pupae, were injected with virus and viral gene expression, as manifested by RNA biosynthesis, was measured using extraction of total RNA by the guanidine method, and followed by hybridization to RNA dot blots. These results (presented in Fig. 5) show preference for virus replication in earlier instars. Gene expression in pupae was minimal.

Virion composition. GmDNV isolated from larvae yields 3 density classes, as seen in Fig. 6 (left, CsCl gradient). This distribution is typical of parvoviruses in general. Furthermore, The 1.32 band is composed of "empty" virions devoid of DNA (Fig. 6, right, alkaline agarose gel). The other two bands contain a uniform species of viral DNA (approximately 6 kb), but the DNA/protein ratios differ between them (table 1). All 3 bands yield 4 polypeptides (90k, 45K, 55K and 62K), at

varying abundance between them, and the 1.32 density class contained 2 additional species of approximately 67-70K (Fig. 7). The In vitro translation of poly (A)-containing RNA in reticulocyte lysate system yielded a prominent 45K polypeptide, indicating that at least this capsid protein is not modified post-translationally.

Legends to Figures

Fig. 1. Clone of GmDNV genome. The 5.4 kbp fragment was cloned in pUC 19 and a restriction map, representing sites for 15 restriction endonucleases, was determined, and shown. The fragment is flanked by sequences from the pUC polylinker (hatched lines). Below, the sequencing strategy is shown. B, BamHI; E1, EcoRI; E5, EcoRV; H, HindIII; Hc , HincII; Hp, HpaI; Xb, XbaI; X, XmnI; Bg, BglII; S, SphI;; N, NcoI.

Fig. 2. Sequences of GmDNV genome. The regions in the sequence marked I-V are the ones denoted in Fig. 1. The inverted terminal repeat is underlinedby one line. A and T stretches of more than 6 residues are underlined by two lines.

Fig. 3. Strand specificity of GmDNV transcription. (a) Structure of pGEM into which fragments from GmDNV genome were inserted. Orientation of riboprobe synthesis is, A and B, from the SP6 promoter; a and b, from the T7 promoter (b) Results of dot blot hybridization. RNA was extracted from injected larvae at day 3 postinjection, dotten onto nitrocellulose filters and hybridized to the ^{32}P-labeled riboprobes described in 3a. M, Mock-injected.

Fig. 4. Size fractionation of GmDNV RNA. Poly (A)-containing RNA from injected larvae and from infected cells was electrophoresed in formamide gels, and transferred to nitrocellulose by the "Northern" blotting procedure. The probe for hybridization was ^{32}P- labeled cloned GmDNV DNA. Size markers were run in separate lanes, and that part of the gel was stained with ethidium bromide. 1, Poly (A)$^-$ RNA; 2, injected larva; 3, infected larva; 4, TN 368 cells infected with GmDNV; 5,6, other lepidopteran cell lines.

Fig. 5. Biosynthesis of GmDNV RNA as a function of larval development. The experiment is described in the text. The graph represents levels of hybridizations of ^{32}P-labeled probe to RNA dot blots.

Fig. 6. GmDNV density classes and their DNA content. Density classes were separated by CsCl centrifugation of virus extracted from virus-infected larvae. To examine virion DNA, an alequote from the virus preparation was placed directly onto an alkaline agarose gel, and viewed by ethidium bromide staining.

Fig. 7. In vitro translating of GmDNV RNA. Viral poly $(A)^+$ RNA was translated in a reticulocyte lysate system, and electrophoresed in a gradient SDS-PAGE. Lanes 1, no RNA; lanes 2-4, increasing amounts of viral RNA; U,M,L, upper, middle and lower bands from CsCl, respectively. M, size markers; C, control without viral RNA; 1,2,3, in vitro translated poly $(A)^+$ RNA from injected larva. U,M,L, Upper, Middle and Lower CsCl density classes of GmDNV.

Fig. 1

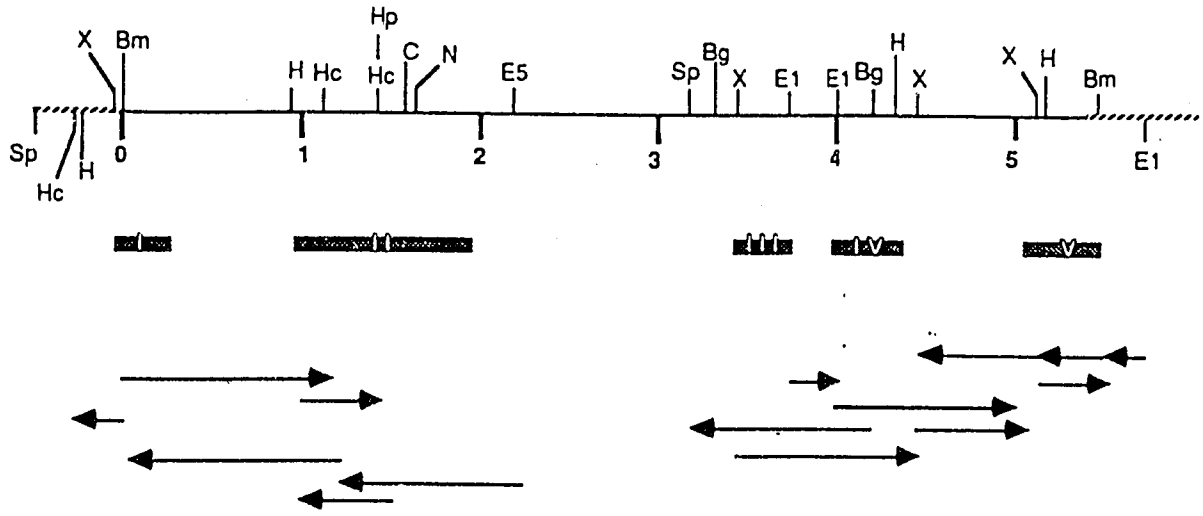

Fig. 2

A + T 1491 (60.8)
C + G 893 (36.4)

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        10        20        30        40        50        60        70        80        90       100       110       120       130
CCTAGCCTTCTGGAACCTCGACTTCCACCTTGTAGTACCTGCACTACAATCATTTCCTGTCATTGTCACACGCTCACTCTATCGTTATGTTGTTAATAATCTTTCTCTATCGTTATACTCACTTGGCTCT

       140       150       160       170       180       190       200       210       220       230       240       250       260
ATTGTACTTTTGTTTGAACTCTATTAAATACGCGTAAAATAATAGAACAATAACACTGGAGTAAACTGGCCGTTGAGCAGCTAGCATTCGATTTGGCACACGTTTTATCCTACACTGACCGGTCGCTGCA

       270       280       290       300       310       320       330       340       350       360       370       380       390
TCTCATATATC..........CTGAGTACAGCTAAGTAGTCGAGCAGTACATGTATAGCGTAGCTCGTGTATGCTATATTTAAGAACACCTGGTAACGCTTTGGTGCTGCTATTGGAGCTATTGGAATTG

       400       410       420       430       440       450       460       470       480       490       500       510       520
GAACTAAGCAAGCTATTGAAAATATAGTGAGTAATCTACCCTTCTGTTTCAGGTATGTCCCGTCATATTAATCCTAGATACATTAATCAACCTAATTGGAAAGATTATATTGCTGAAGAAATTCTAAAAA

       530       540       550       560       570       580       590       600       610       620       630       640       650
TTGGGTCGGATATTCCAATTTACCTGACGATTTTTTTCAAGAAGAAACTCTTTCAGATTCACCGATGAAGAAGCA..........GTTAACGTTAATTCACAAAGTACCGATCCTCAAAATCCTAGTTCA
                                                                                        HpaI

       660       670       680       690       700       710       720       730       740       750       760       770       780
TCTGGTGCTACAACTGATCTTGACGTTACAATGGCTATGTCATTGCCAGGAAACTGGCTCTGGAACATCATCTGGAGGAGGAAACACGCAAGGTCAAGACGTGTATATAATTCCTAGACCATTTTCAACT

       790       800       810       820       830       840       850       860       870       880       890       900       910
TTGGTAAAAAATTAAGTACTTATACTAAATCTCATAAATTTATGATATCGGTTTAGCAAATAATGTAATAGGTCTACCGGAACTGGTACTACTGCTGTAAATCGATTACTTACTACTGTTAGCTGAATAC

       920       930       940       950       960       970       980       990      1000      1010      1020      1030      1040
ATGGCAAAATTACCATTGTATATGAATCAATCAGGAATTTGATTTATTACTCTGGTTCAGAGTGTGAGTGTATGTTAAGTATTTCGTACCTAACGCATGCATTGAACTAGTCTACGTACTAACAGCTACC

      1050      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
TAATCAATTCTACTGCACGCTATGTATATAGTGGATACGGCATCGCTTCACTGTCACATAGATCACTGCACTCGCCTAGTGACGTCGG..........TCTAGATTATGGATATTTCATTGAAGAAATAA
                                                                                          XbaI

      1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
ATTGAGCATATGGATTAAGTTCTTTTTCATCAGTAAAATTATAATCTTCAGTTAGTAAATC TAAATTTCGACGTTAAAGGACATAGCATTGAGATCT..........CTCCAATAACAATAAGAGATAT

      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
TCAAGCTCCGTGTGTGTTATGTCTCTAACAGGTTTTTTTGTAAACATCCTAATAGTAGACATTAATTACAGATGAACAATGGAGACAGCAACAGAGAAACCAATAATACTACCAGATCTAATCAAGATAA

      1440      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
TATACGAGAGTCATCAGGAGAATCCTCTAGTGAACAATGTAGCATGGTGGCAACTTCATCTAGAAAACGTGAGTGGCATTTGGAGGACGAGGAACAATGCCAGCTTGCAAAAGAATCTCAAGAAAACTTT

      1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680      1690
CAATATATGGCAGAAGAATTGGAAAAAATGGGCAGT..........GCAGGCAACGCCAGAGGCCAAAAAAAGCAGGAGTTTCAACAGCAGGAGAATCAGTCCTTGCGTTTCGTTGCTTCTTGCATCGGT

      1700      1710      1720      1730      1740      1750      1760      1770      1780      1790      1800      1810      1820
GAATCTGAAAGAGTTTCTTCTTGAAAAAAAATCGTCAGGTAAATTGAATATCCGACCCAATTTTTAGAATTTCCTTCAGCAATATAATCTTTCCAATTAGGTTGATTAATGTATCTAGGATTAATATGAC

      1830      1840      1850      1860      1870      1880      1890      1900      1910      1920      1930      1940      1950
GGGACATACCTGAACAGAAGGGTAGATTAACTCCACTATATTTTTTCAATAGCTTCCTTAGTTCCAATTCCAATAGCTCCATAGCAGCACCAAAGCTACAGGTGTTCTTAATATGCATACCACGAGCTAC

      1960      1970      1980      1990      2000      2010      2020      2030      2040      2050      2060      2070      2080
GTTAACT..........TCTAGACCATTAACCTCTAGCACATTTATAAGCTTCATAAAATAGAGACTTAGGATTGTATACTTTTTCAAATAAACGGCGAACATAAAGAAATTTTGCCTATAGACATACCT
                  XbaI

      2090      2100      2110      2120      2130      2140      2150      2160      2170      2180      2190      2200      2210
ATTAGAGTTTCTGGAACCACTCGCAATATCACTAGGATCACTGGAAATCACTTATATACTGTCTGTAGACTGATGATGCGCACTCTTTATCTGCTATATACTCTACGTCGCTGGCCAGTCACATCCTATT

      2220      2230      2240      2250      2260      2270      2280      2290      2300      2310      2320      2330      2340
TTGCACACGGTTTAGCTTACGATCGACGAGTTGCCGGTCAAATGAGGTCACAATAACAAGATAATAAAATGCGCATAAATTATCTCAAGTTTGTTTTCATGTTATCTCGGTTCACTCATATTGCTATCTC

      2350      2360      2370      2380      2390      2400      2410      2420      2430      2440      2450
TTTCTAATAATTGTTGTATTGCTATCTCACTCGCACACTGTTACTGTCCTTTACTAACATCAGGTCCATGATGTTCCACCTTCAGCTCCAAGGTCTTCGGATCC.........
                                                                                                  BamHI
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I

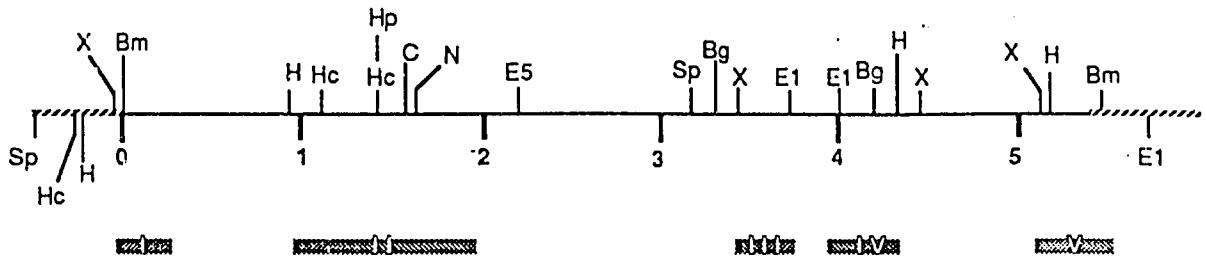
II

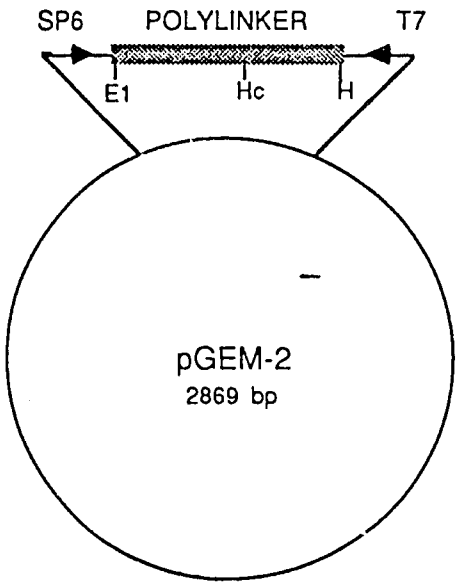
III

IV

V

Fig. 3 a





$H_{0.9} \xleftrightarrow[a]{A} Hc_{1.1}$

$E1_{4.0} \xleftrightarrow[b]{B} H_{4.3}$

Fig. 3 b

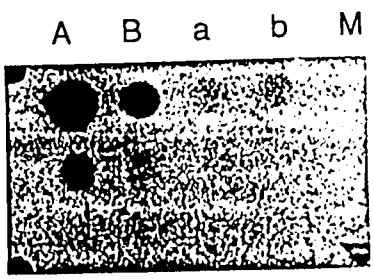


Fig. 4

GmDNV RNA IN INFECTED LARVAE
AND LEPIDOPTERAN CELL LINES

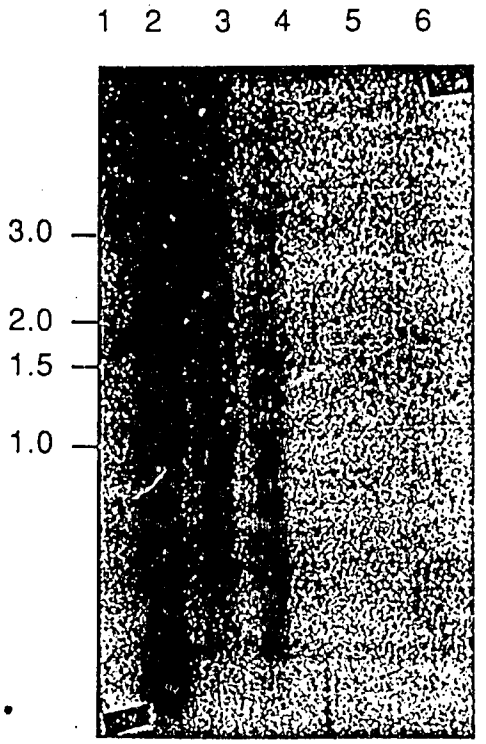


Fig. 5

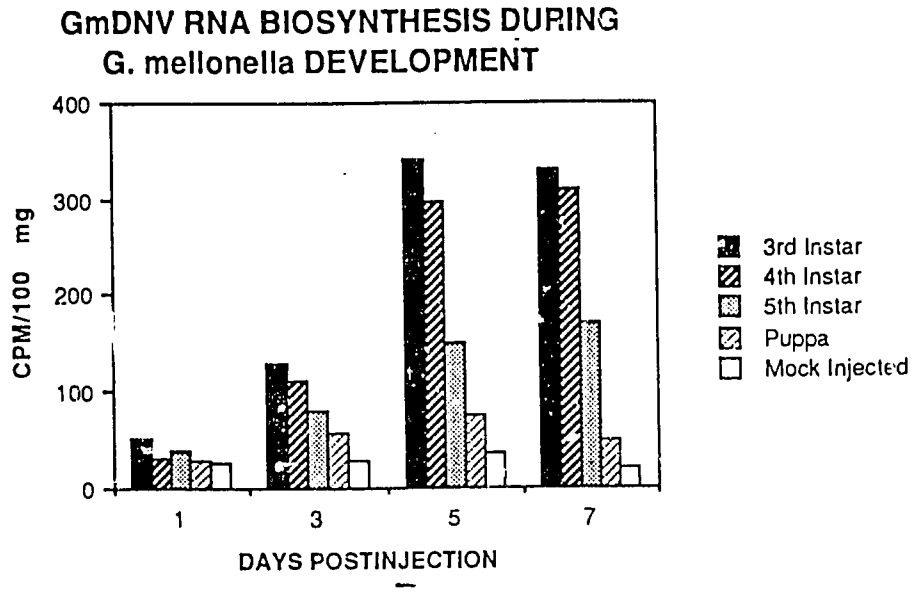

GmDNV RNA BIOSYNTHESIS DURING
G. mellonella DEVELOPMENT
CPM/100 mg
400
300
200
100
0
1
3
5
7
DAYS POSTINJECTION
3rd Instar
4th Instar
5th Instar
Puppa
Mock Injected

Fig. 6

DNA CONTENT OF GmDNV DENSITY CLASSES

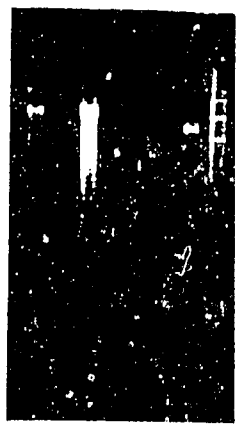
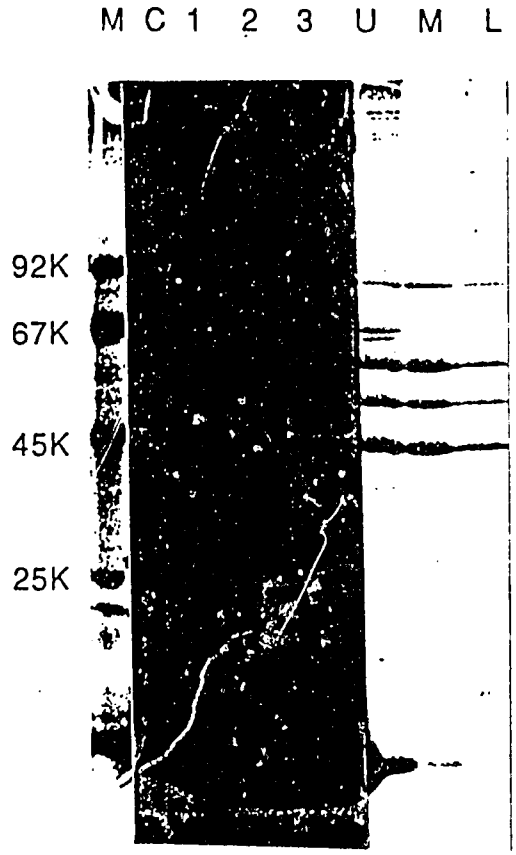


Fig. 7

IN VITRO TRANSLATION OF GmDNV RNA



Conclusions and Discussion

The sequence of about 50% of the genome, and the structural examination of its terminai, revealed a surprising resemblence of the GmDNV genome to that of the human parvoviruses, AAV and B19. Furthermore, the structure of the termini (identical palindromic sequences) raise the interesting possibility that, like the human AAV and B19, GmDNV will be shown to have integration capacity into the host genome. We hope to be able to look into this possibility during the 3rd year, but that depends on our progress in finding a suitable tissue culture system. A major difference, however, is the inverted structures on both termini.

Despite the difficulties posed by the absence of a tissue culture host system, we are progressing in the analysis of the viral transcription products in vivo. Mapping them, as well as mapping transcription signals (promoters, polyadenylation signals, splice domains) will be greatly accelerated once the sequence is (at least nearly) complete.

The study of the replication in heterologous larvae is underway. We have been able to obtain virus from GmDNV-injected Heliothis larvae and we are currently investigating whether the isolate is indeed GmDNV. If it is, it would be intriguing to find out whether it is identical to the wild type, or it is a mutated virus (crossing of species and tissue barriers by mammalian parvoviruses, as a result of a small number of changes in the viral DNA, is a well documented phenomenon). If we have a mutant, we will be facing a problem in mapping the host range mutation in that marker-rescue experiments are not feasible without tissue culture. In that case we may consider sequencing the entire genome. Replication in other species (Spodoptera, Bombyx) is currently being investigated as well.

Expression in tissue culture. We will try to increase the levels of expression by various means. Primarily, DNA transfection, using DEAE Dextran, will replace the infection method, with the hope that the low yields of viral DNA and RNA result from membranal barriers. Also, additional cell lines will be obtained (from Dr. Dwight Lynn, USDA, Beltsville, Maryland) and screened for viral gene expression.

Vector construction. This part will be initiated as soon as we obtain a rough idea about the transcription organization. Initial experiments will include the insertion of the prokaryotic CAT gene under the control of the viral promoter(s), and testing their activities. Alternatively, since our expression studies are conducted in vivo, primarily, the use of the luciferase gene may prove to be a better choice, since the assay, based on the emission of light, is more suitable for in vivo studies.