DNA Sequence Homology Relationships Among Six Lepidopteran Nuclear Polyhedrosis Viruses

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SUMMARY

The DNA sequence homology relationships among six lepidopteran nuclear polyhedrosis viruses (NPVs) have been explored by hybridization of 32P-labelled NPV DNAs to Southern blots of restriction endonuclease-digested NPV DNA. The Autographa californica NPV (AcMNPV) shows extensive DNA sequence homology throughout the entire genome with the Rachiplusia ou NPV (RoMNPV). Both the Orgyia pseudotsugata NPV (OpMNPV) and the Porthetria dispar NPV (PdMNPV) share homologous regions, equivalent to 1% of the DNA genome, with AcMNPV and RoMNPV. This homology is localized in two regions on the AcMNPV physical map although other regions are also weakly homologous. Approx. 1% of the DNA of OpMNPV and PdMNPV show sequence homology with each other; the homology is primarily localized in two to four regions of the genomes. Heliothis zea NPV (HzSNPV) and Trichoplusia ni NPV (TnSNPV) share less than 0.2% sequence homology with the MNPVs and share less than 0.2% sequence homology with each other.

INTRODUCTION

Nuclear polyhedrosis viruses (NPVs) are members of a group of pathogenic invertebrate viruses known as baculoviruses. The viruses replicate in the nuclei of infected host cells and have enveloped, rod-shaped, DNA-containing nucleocapsids which may be enclosed in crystalline protein occlusion bodies known as polyhedra (Smith, 1976). Two types of NPVs are distinguished morphologically on the basis of the number of nucleocapsids per virus envelope within polyhedra: SNPVs have a single nucleocapsid per envelope whereas MNPVs may have multiple nucleocapsids per envelope.

The DNA genomes of baculoviruses are double-stranded, circular, supercoiled molecules with mol. wt. of approx. 60 x 10^6 to 100 x 10^6 (Burgess, 1977; Summers, 1977). Over 98% of the DNA sequences of an NPV genome are unique sequences (Kelly, 1977; Rohrmann et al. 1977; Jurkovicova et al. 1979). Restriction endonuclease fragment patterns of NPV DNA are useful in identifying different NPVs (Miller & Dawes, 1978a) and in recognizing closely related genotypic variants of NPVs (Lee & Miller, 1978; Miller & Dawes, 1978b; Summers & Smith, 1978).

Information concerning the DNA sequence homology among NPVs is very limited. The sequence homology relationships among several MNPVs isolated from closely related species within the genus Spodoptera (Lepidoptera; Noctuidae) were determined; sequence homology ranging from 11 to 75% was reported for various pairs of these viruses (Kelly, 1977; Knudson & Tinsley, 1978). Another study (Rohrmann et al. 1978) indicated that a
SNPV and MNPV isolated from *Orgyia pseudotsugata* shared less than 1% sequence homology. Sequence homology between *Adoxophyes orana* SNPV and *Barathra brassicae* MNPV was also less than 1% (Jurkovicova et al. 1979). There is no sequence homology information available on the relationship of MNPVs isolated from insects of diverse genera or the relationship between different SNPVs.

Our laboratory has undertaken an extensive molecular genetic analysis of the *Autographa californica* MNPV (AcMNPV) which is amenable to genetic characterization in cell culture (Lee & Miller, 1978, 1979). A physical restriction map of the AcMNPV DNA has been constructed (Miller & Dawes, 1979) and it was of interest to determine sequence homology relationships between AcMNPV and other NPVs, correlate sequence homology with specific regions of the AcMNPV map and, if possible, correlate map regions with host-range properties of the NPVs studied. In addition to fundamental virological and evolutionary interest, the research is of considerable applied interest in relationship to the use of this virus as a microbial insect control agent (Summers et al. 1975). Three NPVs are currently registered by the U.S. Environmental Protection Agency (U.S.E.P.A.) as pesticides: the *Heliothis zea* SNPV (HzSNPV), the *O. pseudotsugata* MNPV (OpMNPV) and the *Porthetria dispar* MNPV (PdMNPV). These viruses were thus obvious candidates for the nucleic acid sequence homology studies described here. In addition, *Trichoplusia ni* SNPV (TnSNPV) was chosen to explore SNPV homology relationships and *Rachiplusia ou* MNPV (RoMNPV) was selected for comparison with AcMNPV due to shared host-range and other reported similarities (Harper, 1976; Cibulsky et al. 1977; Smith & Summers, 1978; Summers & Smith, 1978).

The Southern blot technique for transferring DNA from agarose gels to nitrocellulose filter paper (Southern, 1975) was employed so that different regions of the NPV genomes could be individually tested for sequence homology. Furthermore, the technique enhances the sensitivity of detecting very low levels of sequence homology (0.2 to 1%) at specific regions of the genome which are obscured by background hybridization using more conventional filter hybridization techniques.

**METHODS**

*Viruses.* The HzSNPV was originally provided by Dr C. Ignoffo (USDA, Colombia, Mo., U.S.A.) as a commercial pesticide preparation (Viron/H, IMC, Libertyville, Ill., U.S.A.) and was subsequently propagated in *H. zea* larvae. The TnSNPV was originally provided by Dr R. P. Jacques (Harrow, Ontario) and was subsequently propagated in *T. ni* larvae. The RoMNPV was provided for us by Dr J. D. Harper (Auburn University, Auburn, Ala., U.S.A.) and subsequently propagated in *T. ni* larvae in our laboratory. The virus sample received from J. D. Harper was a few passages from the original RoMNPV sample sent to him by J. Paschke. The virus was passaged three times in our laboratory for use in this research. The PdMNPV was directly provided by Dr Martín Shapiro (USDA, Otis AFB, Mass., U.S.A.) as infected *P. dispar* larvae. The OpMNPV was isolated from *O. pseudotsugata* larvae collected during the Kamloops (B.C., Canada) infestation of 1976. Restriction endonuclease fragment patterns of this OpMNPV have been previously published and the similarity of the Kamloops virus isolate and the OpMNPV registered as a pesticide by the U.S.E.P.A. was established (Miller & Dawes, 1978a). The AcMNPV L-1 variant was used in these studies. The isolation of this variant has been described (Lee & Miller, 1978) and a physical map of restriction endonuclease sites of the variant has been determined (Miller & Dawes, 1979). The AcMNPV was propagated in *T. ni* larvae as previously described (Lee & Miller, 1978).
DNA homology among NPVs

Virus purification. Occluded viruses were purified from diseased insect larvae as previously described (Miller & Dawes, 1978a) with minor modifications. Centrifugation through 40 to 65% sucrose gradients was carried out at the reduced speed of 17000 g for 30 min. The PdMNPV was unusually difficult to purify from insect debris and additional intermediate NaCl, SDS and 4 M-urea washes outlined by McCarthy & Liu (1976) were utilized.

Isolation of NPV DNA. The NPV DNAs were isolated from occluded viruses by phenol extraction following alkali disruption (Miller & Dawes, 1978a). The isolation of PdMNPV DNA was similar to HzSNPV DNA purification in that the PdMNPV isolation also required additional sucrose isopycnic gradient purification of alkali-liberated virions before SDS treatment and phenol extraction.

Isolation of insect DNA. Spodoptera frugiperda DNA was provided by Mr Scott Franzblau and was prepared from S. frugiperda (IPLB-SF-21) cells grown in suspension in TC-100 media (Lee & Miller, 1978). Purification of this cellular DNA included phenol extraction.

Restriction endonucleases and DNA digestion. EcoRI, HindIII, BamHI and XmaI were purified in our laboratory and tested for purity. DNA digestions were carried out as previously described (Miller & Dawes, 1978a) except that XmaI digestions were performed at 50 °C rather than 37 °C.

Gel electrophoresis. Horizontal slab 0.7% agarose gels were totally submerged in B buffer (Miller & Dawes, 1978a). DNA was loaded into preformed slots along with 5% sucrose and tracer amounts of bromophenol blue. A constant current of approx. 75 mA was applied through the B buffer and gel for approx. 15 h, at which time the marker dye had reached the bottom of the agarose gel. DNA fragments were visualized by ethidium bromide staining under u.v. light (Miller & Dawes, 1978a).

Southern blotting and DNA hybridization conditions. Transfer of DNA from gels to nitrocellulose filters was achieved by the Southern technique (Southern, 1975) and strips of the filters were hybridized with 32P-labelled probe DNA under conditions similar to those previously described (Miller & Dawes, 1979). In brief, the filter strips were pre-incubated in Denhardt’s mixture and 1 x 10^5 ct/min of heat-denatured probe DNA in 3 x SSC was then added. Incubation proceeded for 20 h at 55 or 65 °C as indicated in the text. The strips were subsequently washed three times, 15 min each time, in 250 ml of 3 mM-tris at room temperature. Autoradiographic procedures were similar to those previously described, although times of exposure varied as indicated.

In vitro 32P-labelling of DNA. Virus DNA and insect DNA were radioactively labelled by nick translation (Miller & Dawes, 1979) and prepared for use in hybridization studies by phenol extraction and dialysis. The specific activities of the DNAs were all initially similar (approx. 6 x 10^7 ct/min/μg) except for the OpMNPV DNA which had a specific activity approximately one-third that of the other labelled DNAs.

Estimation of relative sequence homology. In the case of DNAs sharing little or no sequence homology, the percent of shared sequence homology was estimated by comparing the intensity of autoradiographic exposure produced by a small, completely homologous fragment with the intensity of the bands produced by the less homologous DNA. For example, on blots of HindIII-digested AcMNPV DNA the intensity of the small fragment Hind-U (Miller & Dawes, 1979) hybridized to 32P-labelled AcMNPV probe DNA provides an indication of the intensity of exposure expected for a band of DNA with approx. 0.7% sequence homology. The difference in specific activity of the labelled OpMNPV compared to the specific activities of the other DNAs was taken into account. Estimation of sequence homology by this method is roughly quantitative and cannot be considered precise. It is important to note that the Southern blot hybridization method employed in this research is most useful for detecting regions of the DNA with sequence homology. The employed
hybridization methods indicate that a region of the DNA has or does not have some sequence homology but do not determine the percent of matched base pairs within that region. Determining the percent sequence homology in terms of the percent of matched or mismatched nucleotide base pairs within a homologous region requires more rigorous control and variation of the hybridization conditions.

RESULTS

Comparative restriction endonuclease fragment patterns of the six NPV DNAs

Agarose gel electrophoresis of the DNA fragments generated by HindIII digestion of the six NPV DNAs is presented in Fig. 1. All six NPV DNAs show distinctive fragment patterns which may be used for identification purposes (Miller & Dawes, 1978a). Fragment patterns of EcoRI-digested NPV DNAs are shown in Fig. 2. Again, each NPV DNA shows a distinctive fragment pattern but the similarity between the AcMNPV pattern and the RoMNPV pattern is more apparent, particularly in the lower regions of the gel where many fragments co-migrate. The similarity between AcMNPV and RoMNPV are further illustrated in Fig. 3 where the BamHI, SsrI and SalI patterns are compared. The AcMNPV and RoMNPV are indistinguishable by XmaI digestion (data not shown). The BamHI patterns show co-migration of four RoMNPV fragments with four of the seven AcMNPV BamHI fragments. The SsrI patterns show marked similarities between the two virus DNAs with about half the fragments co-migrating.

Hybridization of NPV DNA probes to AcMNPV and RoMNPV blots

The transfer of DNA from gels to nitrocellulose filters, maintaining the original fragment pattern, is accomplished by the Southern blot procedure (Southern, 1975). In Fig. 4, HindIII-digested AcMNPV DNA was blotted on to a sheet of nitrocellulose and longitudinal strips of the blot were individually incubated with ³²P-labelled fragments of five different NPV DNAs at 65 °C in Denhardt’s mixture. Following hybridization, the strips were washed and X-ray films were exposed for 4 days (Fig. 4a) or 16 days (Fig. 4b). As expected, ³²P-labelled AcMNPV probe DNA hybridizes to each of the HindIII fragments of the AcMNPV blot (compare Fig. 1 and 4). RoMNPV probe DNA also hybridizes to each of the AcMNPV HindIII fragments (Fig. 4) even though many of the RoMNPV HindIII fragments do not co-migrate with AcMNPV HindIII fragments (Fig. 1). All regions of the AcMNPV DNA thus have homologous regions with the RoMNPV genome. No hybridization between ³²P-labelled TnSNPV or HzSNPV probes and the AcMNPV blot was detected even by over-exposure of the gel (Fig. 4b). The technique employed is sufficiently sensitive to detect approx. 0.2% sequence homology localized in one fragment. Hybridization of these probes to AcMNPV HindIII blots under the less stringent hybridization temperature of 55 °C did not reveal any less conserved homology regions (data not shown).

Very weak hybridization of the OpMNPV probe to several of the large AcMNPV HindIII fragments is detected upon extensive over-exposure. Owing to difficulties in photographic reproduction of autoradiograms, this faint hybridization may not be observable in Fig. 4(b). The OpMNPV probe hybridized to AcMNPV HindIII-D or E, HindIII-H and more weakly to other fragments (see Miller & Dawes, 1979 for fragment lettering and the AcMNPV physical map). The amount of hybridization is not more than 0.5% taking into account that the specific activity of the OpMNPV probe was approx. one-third the specific activity of the other NPV probes used.
DNA homology among NPVs

A R T H O P

Fig. 1. Gel electrophoresis of the HindIII digestion products of the six NPV DNAs. Each of the six NPV DNAs was digested to completion with HindIII restriction endonuclease and approx. 2 μg of the resulting DNA fragments were loaded into slots of a 0.7% agarose horizontal slab gel. As mol. wt. markers, HindIII-generated λ bacteriophage DNA fragments were loaded into the outer gel slots. Following electrophoresis, the gels were stained with ethidium bromide and photographed under u.v. light. The DNA in each lane is indicated at the top of the lanes as follows: λ bacteriophage DNA; A, AcMNPV DNA; R, RoMNPV DNA; T, TnSNPV DNA; H, HzSNPV DNA; O, OpMNPV DNA; P, PdMNPV DNA.

Over-exposed blots of EcoRI-digested AcMNPV DNA, hybridized at 55 °C to each of the six NPV DNA probes as well as an insect DNA probe, are shown in Fig. 5. Each of the AcMNPV EcoRI fragments on the blot hybridizes to both AcMNPV probe DNA and RoMNPV probe, again indicating that homology extends throughout the AcMNPV genome. The TnSNPV DNA, HzSNPV DNA and insect DNA probes show no hybridization to the AcMNPV EcoRI blots, consistent with the results of the AcMNPV HindIII blots of Fig. 4. The insect DNA probe primarily serves as a control in these experiments.
The OpMNPV and PdMNPV DNAs both show very weak hybridization to several AcMNPV EcoRI fragments which may not photographically reproduce. OpMNPV probe hybridizes to the AcMNPV EcoRI-F,G,G' region, the EcoRI-H region, the EcoRI-K or L region and other regions more faintly. The total amount of sequence homology however is very low (approx. 1% total). The very weak hybridization is clearly not due to indiscriminate binding of probe DNA since insect, TnSNPV and HzSNPV DNA probes showed no apparent hybridization.

Over-exposed blots of BamHI-digested RoMNPV DNA are presented in Fig. 6. Both RoMNPV DNA and AcMNPV DNA hybridize to all of the RoMNPV blot fragments. TnSNPV, HzSNPV and insect DNA probes do not hybridize to the RoMNPV fragments.
DNA homology among NPVs

Fig. 3. Comparison of various restriction endonuclease fragment patterns of AcMNPV and RoMNPV DNAs by gel electrophoresis. AcMNPV (A) and RoMNPV (R) DNAs were individually digested with BamHI, SalI, or SstI and electrophoresed through 0.7% agarose gels. λ bacteriophage DNA digested with HindIII was used as a mol. wt. standard.

Both OpMNPV and PdMNPV DNA probes hybridize weakly to the second and third smallest RoMNPV fragment (equivalent to AcMNPV BamHI-F, and BamHI-E respectively; Miller & Dawes, 1979). Weaker hybridization to one or more of the largest fragments is also observed; these RoMNPV fragments are difficult to resolve (see Fig. 3). The results are consistent with the AcMNPV blots. In addition, AcMNPV probe DNA hybridized to EcoRI blots of RoMNPV showed AcMNPV homology with all of the RoMNPV EcoRI fragments (data not shown).
Fig. 4. Hybridization of ³²P-labelled NPV DNA probes to blots of electrophoretically separated HindIII fragments of AcMNPV DNA. HindIII fragments of AcMNPV were transferred from agarose gels to a nitrocellulose sheet by the Southern blot procedure. Strips of the nitrocellulose blot were hybridized at 65 °C to ³²P-labelled NPV DNA probes. The strips were then washed, re-aligned and autoradiographed for (a) 4 days or (b) 16 days. Numbered strips correspond to probes of AcMNPV (1), TnSNPV (2), HzSNPV (3), OpMNPV (4) and RoMNPV (5) DNA. Asterisks on the right indicate AcMNPV fragments sharing sequence homology with OpMNPV DNA.

Hybridization of NPV DNA probes to TnSNPV and HzSNPV blots

In order to explore the relationship between the two SNPVs as well as further determine the relationship of the SNPVs to MNPVs, blots of EcoRI-digested TnSNPV and HzSNPV were hybridized to various NPV and insect probe DNAs and are shown in Fig. 7. The TnSNPV probe hybridized to TnSNPV blot fragments and the HzSNPV probe hybridized to HzSNPV blot fragments as expected. No hybridization of TnSNPV probe to HzSNPV blot fragments or vice versa was observed. Also no hybridization of any MNPV DNA probe was observed on either SNPV blot. Hence TnSNPV and HzSNPV DNA share less than 0.2% sequence homology with any of the other NPVs tested.
DNA homology among NPVs

Fig. 5. Hybridization of 32P-labelled NPV DNA probes to blots of electrophoretically separated EcoRI fragments of AcMNPV DNA. Strips of a blot of EcoRI-digested AcMNPV DNA were hybridized at 55 °C to 32P-labelled DNA probes. Autoradiographic exposure time was 2 weeks. Numbered strips correspond to probes of TnSNPV (1), HzSNPV (2), OpMNPV (3), PdMNPV (4), RoMNPV (5), S. frugiperda cell (6) and AcMNPV (7) DNA. Arrows on the right indicate AcMNPV DNA fragments sharing sequence homology with OpMNPV and PdMNPV DNAs.

Hybridization of NPV DNA to OpMNPV blots

To determine sequence homology between OpMNPV and PdMNPV as well as to investigate further NPV homology relationships, HindIII fragment blots of the OpMNPV DNA were prepared. In Fig. 8, blots of HindIII-digested OpMNPV were hybridized to the six NPV DNA probes and insect DNA probes. No hybridization was observed with the TnSNPV, HzSNPV and insect DNA probes. OpMNPV probe hybridized with all the OpMNPV HindIII fragments as expected. PdMNPV probe hybridized weakly to four
Fig. 6. Hybridization of \(^{32}\)P-labelled NPV DNA probes to blots of electrophoretically separated \textit{BamHI} fragments of RoMNPV DNA. Hybridization of probe DNA to blots of \textit{BamHI}-digested RoMNPV DNA was carried out at 55 °C and autoradiographic exposure time was 3 weeks. Numbered strips correspond to probes of TnSNPV (1), HzSNPV (2), OpMNPV (3), PdMNPV (4), \textit{S. frugiperda} cell (5), RoMNPV (6) and AcMNPV (7) DNA. Asterisks on the right indicate RoMNPV fragments sharing sequence homology with OpMNPV and PdMNPV DNA.

regions of the gel denoted by the numbers 1 to 4 on the right of the diagram. Hybridization in these four regions of the gel was also observed under the more stringent hybridization temperature of 65 °C (data not shown). The strongest hybridization was in band number 4. Extremely faint hybridization was observed in other regions of the gel. The AcMNPV and RoMNPV DNA probes hybridized very faintly to a number of the OpMNPV bands including bands 1 to 4.

Hybridization of the NPV DNA probes to \textit{HindIII}-digested PdMNPV blots showed consistent hybridization results: the PdMNPV DNA probe hybridized to all \textit{HindIII} PdMNPV fragments, the HzSNPV, TnSNPV and insect DNA probes showed no hybridization, the AcMNPV and RoMNPV DNA probes weakly hybridized to several PdMNPV fragments and the OpMNPV DNA probe weakly hybridized to one band and several others more weakly (data not presented).
DNA homology among NPVs

Fig. 7. Hybridization of 32P-labelled NPV DNA probes to blots of electrophoretically separated EcoRI fragments of (a) TnSNPV DNA and (b) HzSNPV DNA. Strips of the blots were hybridized to probe DNA at 55 °C and autoradiographic exposure time was 2 weeks. Numbered strips correspond to probes of AcMNPV (1), TnSNPV (2), HzSNPV (3), OpMNPV (4), PdMNPV (5), RoMNPV (6) and S. frugiperda cell (7) DNA.

DISCUSSION

Hybridization to Southern blots of restriction endonuclease (REN)-digested NPV DNA provides a powerful technique for exploring sequence homology relationships among NPV DNAs. More detailed information has been gained concerning the relationship of two closely related viruses (AcMNPV and RoMNPV) and between very distantly related viruses (e.g. AcMNPV, PdMNPV and OpMNPV). A lack of sequence homology (less than 0.2%) was observed between two SNPVs and the other MNPVs studied.

A close relationship between AcMNPV and RoMNPV was previously noted by protein analysis (Cibulsky et al. 1977; Summers & Smith, 1978), comparison of EcoRI fragment patterns (Smith & Summers, 1978) and host-range studies (Harper, 1976). More differences are observed between the REN fragment patterns of the AcMNPV and RoMNPV than between the REN patterns of AcMNPV and TnMNPV (Miller & Dawes, 1978b). In some cases, only approx. one-half of the REN fragments of the AcMNPV and RoMNPV DNAs
Fig. 8. Hybridization of $^{32}$P-labelled NPV DNA probes to blots of electrophoretically separated $HindIII$ fragments of OpMNPV DNA. Strips of the blot were hybridized at 55 °C to probe DNA and autoradiographic exposure time was 2 weeks. Numbered strips correspond to probes of TnSNPV (1), HzSNPV (2), OpMNPV (3), PdMNPV (4), S. frugiperda cell (5), RoMNPV (6) and AcMNPV (7) DNA. The numbers on the right refer to fragments of OpMNPV on the blot to which PdMNPV hybridizes.

Fig. 9. The physical map of AcMNPV (Miller & Dawes, 1979). The map is presented in linear form; map units expressed $\times 10^{-8}$. 

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Fig. 8. Hybridization of $^{32}$P-labelled NPV DNA probes to blots of electrophoretically separated $HindIII$ fragments of OpMNPV DNA. Strips of the blot were hybridized at 55 °C to probe DNA and autoradiographic exposure time was 2 weeks. Numbered strips correspond to probes of TnSNPV (1), HzSNPV (2), OpMNPV (3), PdMNPV (4), S. frugiperda cell (5), RoMNPV (6) and AcMNPV (7) DNA. The numbers on the right refer to fragments of OpMNPV on the blot to which PdMNPV hybridizes.
co-migrate in gels (e.g. *BamHI, HindIII*, and *EcoRI*). Such differences in REN fragment patterns might arise by random point mutation which, in the course of evolution, would occasionally affect REN recognition sites, or by recombination with a more distantly related virus giving rise to a hybrid virus containing homologous and non-homologous regions. These two possibilities can be distinguished by determining if all regions of the two DNAs are homologous (point mutation origin) or if only those fragments that co-migrate are homologous (recombination origin). Hybridization of AcMNPV and RoMNPV DNA probes to various Southern blots of REN-digested AcMNPV and RoMNPV DNA demonstrate that all regions of the AcMNPV DNA genome share sequence homology with the RoMNPV DNA genome and vice versa. Hence, the two viruses are probably related to each other primarily by random point mutation although other more complex events such as inversion and limited gene duplication may have occurred in the evolutionary divergence of the two genomes. Nevertheless, on the basis of the extensive sequence homology relationships demonstrated here, RoMNPV and AcMNPV may be classified as variants.

The hybridization methods employed in this research assess whether specific regions of two different virus DNA genomes share some sequence homology but the methods employed do not reveal whether those regions which share sequence homology are totally (i.e. 100%) homologous at the nucleotide level. Thus, AcMNPV and RoMNPV share homology throughout their genomes but they are not 100% homologous, as is evident from REN analysis. The percent nucleotide sequence homology between AcMNPV and RoMNPV can be directly determined by altering the stringency of hybridization. The fraction of base substitutions between the two DNA genomes may be estimated from REN data using the previously derived equation $p = 1 - \sqrt{F^2 + 8F}/2$ where $F$ is the fraction of conserved REN sites, $n$ is the number of base pairs per cleavage site and $p$ is the estimated number of base substitutions per base pair by which the two DNAs differ at the sites compared (Upholt, 1977). Since AcMNPV and RoMNPV have in common roughly half of the sites recognized by RENs with hexanucleotide specificity, the estimated frequency of base substitutions per nucleotide at the cleavage sites compared between AcMNPV and RoMNPV is approx. 0.04. If the cleavage sites are representative of the remaining nucleotide sequences, a rough estimate of overall nucleotide sequence homology between AcMNPV and RoMNPV is 96%. Since AcMNPV and RoMNPV have similar sequence homology relationships with the other four NPVs studied, the remaining discussion will refer to only AcMNPV.

Hybridization of OpMNPV and PdMNPV DNA probes to AcMNPV blots revealed sequence homology between AcMNPV and OpMNPV and between AcMNPV and PdMNPV representing approx. 0.5 to 1.0% of the DNA genomes. This amount of homology is equivalent to slightly less than one common gene assuming an average gene size of 0.8 × 10^6. The AcMNPV fragments with sequence homologous to OpMNPV were *HindIII-H, HindIII-D* or E, *EcoRI-F, G or G* and more weakly *EcoRI-K* or L (see physical map in Fig. 9). In addition, the *BamHI-F* and -E apparently have some homologous sequences and possibly other regions of weaker homology occur as well. Correlation of this data with the physical map of the AcMNPV DNA genome indicates that there are at least two specific regions of DNA sequence homology. One region, between 59.5 and 61.7 map units, includes all of *BamHI-E* and portions of *HindIII-H* and *EcoRI-G*. The other region, between 80.9 and 82.2 map units, includes all of *BamHI-F* and a portion of *EcoRI-H*. Several very small *HindIII* fragments lie in this region and homology with these fragments would be more difficult to detect. These regions appear to contain the most homology although weaker homology in other regions of the genome also exists. Not all the sequences within the two primary regions are homologous since they collectively represent about 4% of the AcMNPV genome.
Specific regions of sequence homology between OpMNPV and PdMNPV DNAs were also observed. Observations of four different fragments with sequence homology suggests that at least two and possibly four or more regions of homology exist between these two DNA genomes. The AcMNPV may also share these regions of homology since AcMNPV also weakly hybridized to these regions although hybridization to regions 3 and 4 was not as intense as in the case of PdMNPV (Fig. 8).

Hence OpMNPV, PdMNPV and AcMNPV all have approx. 1% sequence homology among them. Since OpMNPV and PdMNPV both hybridize to the same AcMNPV regions, it is probable that all three MNPVs share some common regions of homology. The fact that this homology is observed using stringent hybridization criteria (65 °C) indicates that the sequences within these regions are not extensively divergent.

Sequence homology was not observed between the two SNPVs studied nor was any sequence homology observed between an SNPV and an MNPV. The level of detection was approx. 0.2% homology. The lack of sequence homology between TnSNPV and AcMNPV is noteworthy since TnSNPV shares the common host T. ni with AcMNPV and its closely related variant, TnMNPV (Miller & Dawes, 1978b). This situation is thus analogous to the relationship between O. pseudotsugata MNPV and SNPV previously described (Rohrmann et al. 1978). These latter two viruses share the same host yet have less than 1% sequence homology. Interestingly, however, the major occlusion body proteins of these two viruses share common antigenic determinants (Rohrmann, 1977).

It is premature to speculate on whether the sequences common to the AcMNPV, OpMNPV and PdMNPVs include sequences specifying the occlusion body protein (polyhedrin). This gene, however, is a likely candidate since immunological cross-reactivity among polyhedrins of diverse lepidopteran NPVs has been reported (Krywienczyk & Bergold, 1961). Since the function of polyhedrin is primarily to protect the occluded virions in the environment during host organism to host organism transmission (Smith, 1976), there is possibly a lack of host-exerted evolutionary pressure on this protein. The hosts of the MNPVs sharing weak sequence homology are relatively diverse lepidopteran species. Both O. pseudotsugata (the Douglas-fir tussock moth) and P. dispar (the gypsy moth) belong to the family Lymantriidae whereas A. californica (the alfalfa looper) and R. ou (the mint looper) belong to the family Noctuidae.

The significance of the homologous regions of the MNPVs is not yet known but the location of the homologous regions on the AcMNPV physical map may be particularly significant when genetic mutants of AcMNPV are mapped with respect to the physical map (Lee & Miller, 1979). The importance of detecting very low levels of sequence homology in determining the evolutionary relationships among NPVs is emphasized by this research. The Southern blot technique is clearly a powerful tool for exploring sequence homology among viruses.

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