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# Restriction Map of *Rachiplusia ou* and *Rachiplusia ou-Autographa californica* Baculovirus Recombinants

GALE E. SMITH AND MAX D. SUMMERS*

*Department of Entomology, Texas A&M University, College Station, Texas 77843*

The restriction sites of *Rachiplusia ou* nuclear polyhedrosis virus (Ro*M*NPV) DNA were mapped for the endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI. Of the 60 DNA restriction sites of Ro*M*NPV, 35 mapped in similar positions as compared to the restriction sites of *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) DNA. Two plaque-purified viruses, obtained from randomly picked plaques of a wild-type isolate of Ro*M*NPV, were recombinants of Ro*M*NPV and Ac*M*NPV. The recombinants were shown to have Ro*M*NPV and Ac*M*NPV restriction fragments as well as structural polypeptides from each parental virus. Both recombinant viruses had a major Ro*M*NPV capsid protein but were occluded in the Ac*M*NPV polyhedrin protein.

The nuclear polyhedrosis virus of *Rachiplusia ou* (Ro*M*NPV) is an insect baculovirus with a double-stranded, circular, supercoiled DNA genome of about 82 × $10^6$ daltons (7). Previous reports (7, 9-11, 13) demonstrated that Ro*M*NPV is distinct from, but closely related to, *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV). Restriction endonuclease analysis of Ro*M*NPV and Ac*M*NPV DNAs (7) has shown that the genomes are of similar size and that at least 40% of Ro*M*NPV DNA *Eco*RI restriction fragments representing about 50% of the genome have the same mobilities in agarose gel electrophoresis as Ac*M*NPV *Eco*RI fragments. Ac*M*NPV and Ro*M*NPV structural polypeptides when compared by polyacrylamide gel electrophoresis were also very similar (10). Yet the mobilities of several structural proteins are distinct between these two viruses; for example, a major Ro*M*NPV capsid polypeptide of 36,000 daltons is about 1,000 daltons smaller than a major Ac*M*NPV capsid polypeptide (10). Polyhedrin, the protein that makes up the crystalline matrix in which these viruses are often found occluded, is composed of a single polypeptide of about 30,000 daltons for both Ac*M*NPV and Ro*M*NPV. The amino acid sequences of Ac*M*NPV and Ro*M*NPV polyhedrins are similar but unique as determined by tryptic peptide analysis (9).

An elegant technique involving an analysis of viral recombinants has been used to map adenovirus *ts* mutations and the location of templates specifying polypeptides on the physical map of the DNA (3, 14). More recently, many polypeptides and several viral functions of herpes simplex virus types 1 and 2 have been mapped by similar methods (5, 6). This report will initiate studies to use viral recombinants for the mapping of Ac*M*NPV and Ro*M*NPV gene products. The restriction endonuclease cleavage map of Ro*M*NPV DNA will be shown and compared to the cleavage sites mapped for Ac*M*NPV DNA (8). In addition, two recombinants of Ac*M*NPV and Ro*M*NPV were isolated from randomly picked plaques of a wild isolate of Ro*M*NPV. The DNA structure and viral structural proteins of the two recombinants will be presented. In a subsequent report, the physical location of crossover points between Ac*M*NPV and Ro*M*NPV in recombinant genomes will be determined and related to the segregation of those viral proteins that are different between the two parental viruses.

## MATERIALS AND METHODS

**Cells and virus.** A continuous cell line of *Trichoplusia ni*, TN-368, was maintained as described by Volkman and Summers (12) and used for the preparation of viral isolates.

The E2 variant of Ac*M*NPV for which we have prepared a physical map (8) was used in this study. Two wild isolates of Ro*M*NPV were obtained from C. Y. Kawanishi (Environmental Protection Agency, Research Triangle Park, N.C.). Originally, one isolate, designated as Ro*M*NPV wt-1, had been obtained from James Harper (Auburn University). A second isolate, designated as Ro*M*NPV wt-2, was from the Cornell Experiment Station (Geneva, N.Y.). The Ro*M*NPV isolates were plaque purified as described by Summers and Smith (10). Ro*M*NPV R1 was isolated from Ro*M*NPV wt-2. The Ac*M*NPV and Ro*M*NPV recombinants AR7 and AR8 and the Ac*M*NPV variant R9 were isolated from Ro*M*NPV wt-1. Isolation of polyhedra and purification of virus were done as described previously (10).

**Polyacrylamide gel electrophoreses.** To analyze the viral structural polypeptides, purified virus was

disrupted in 1.0% sodium dodecyl sulfate–5.0% 2-mercaptoethanol at 100°C for 3 min. Samples of 30 to 40 μg of viral protein were electrophoresed in 11% polyacrylamide vertical gel slab in the presence of 0.1% sodium dodecyl sulfate as described by Laemmli (1) as modified for baculovirus preparations (10).

**Preparation and purification of $^{32}$P-labeled DNA.** Viral DNA was labeled with radioactive isotope by adding phosphate-free medium (9) containing 50 μCi of carrier-free $^{32}$Pi per ml (50 Ci/mmol; New England Nuclear Corp.) to infected cell monolayers as described previously (8). Viral DNA was isolated by disrupting purified virus with sodium dodecyl sulfate and protease K followed by phenol extraction (8). About $5 \times 10^6$ cpm of viral [$^{32}$P]DNA with a specific activity of $0.5 \times 10^6$ to $1.0 \times 10^6$ cpm/μg was recovered per $10^7$ infected cells.

**Restriction endonucleases and agarose gel electrophoresis.** *Bam*HI, *Eco*RI, *Kpn*I, *Sac*I, *Sma*I, and *Xho*I restriction endonucleases were purchased from New England Biolabs (Beverly, Mass.). All restriction enzyme digests were done as recommended by the supplier with demonstrated excess enzyme to achieve a limit digestion. λ DNA (0.5 μg) was added to all [$^{32}$P]DNA digests as an internal standard to confirm that a limit digest had occurred.

Horizontal agarose gel electrophoresis and autoradiography were done as described previously (8).

**Isolation and digestion of DNA restriction fragments from Sea Plaque agarose.** Isolation of DNA restriction endonuclease fragments was carried out by the method of Parker and Seed (R. Parker and B. Seed, Methods Enzymol., in press). Low-melting-temperature agarose gels were made from Sea Plaque agarose (Marine Colloids, Rockland, Maine). Horizontal gels of 0.6% were made by dissolving the agarose at 100°C in E buffer (40 mM Tris, 5 mM NaOAc, 1 mM EDTA, 0.5 μg of ethidium bromide per ml adjusted to pH 7.4 with glacial acetic acid). About 1 μg of [$^{32}$P]DNA restriction fragments were electrophoresed in each slot at 75 V for 36 to 48 h. The UV visible bands were excised from the gel with a razor blade and stored at −70°C.

DNA restriction fragments recovered from Sea Plaque agarose gels were incubated at 70°C to melt the agarose without denaturing the DNA. The samples were allowed to equilibrate to 37°C, at which the low-melting-temperature agarose remained in solution. Ten microliters of [$^{32}$P]DNA restriction fragments was added to 20 μl of an enzyme digestion solution to give a final concentration of 1.0× restriction endonuclease buffer, 0.1 mg of bovine serum albumin per ml, 0.5 μg of λ DNA, and 2 U of enzyme (1 U of enzyme digest and 1 μg of λ DNA to completion in 15 min). The samples were incubated for 3 h at 37°C, and the reactions were terminated by adding 3 μl of a solution containing 0.1 M EDTA, 1.0% bromophenol blue, 1.0% sodium dodecyl sulfate, and 40% glycerol. The DNA samples, still in a solution of Sea Plaque agarose, were layered directly into the wells of the second gel and electrophoresed as described above.

**Tryptic peptide mapping of polyhedrin.** Virus polyhedra labeled with $^{32}$P were purified from $2 \times 10^7$ infected TN-368 cells at 72 h postinfection as described previously (7). The $^{32}$P-labeled polyhedrin protein was released by disrupting the polyhedra in 0.1 M $Na_2CO_3$–0.17 M NaCl–0.001 M EDTA (pH 10.8) and separated from the virus by centrifugation at 100,000 × *g* for 30 min. The supernatant containing the phenol-soluble polyhedrin protein (9) was extracted twice with redistilled phenol and then precipitated from the phenol phase by adding 3 volumes of cold acetone and incubating at −20°C overnight. The precipitated polyhedrin was pelleted at 10,000 × *g* for 30 min and washed twice with distilled water, and the pellet was resuspended in 0.05 M $NH_4HCO_3$. TR tolylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (Worthington Biochemicals Corp., Freehold, N.J.) was added at an enzyme-to-substrate ratio of 50:1. Polyhedrin was digested at 37°C for 18 h, lyophilized, and resuspended in 10 to 20 μl of 0.05 M $NH_4OH$. About 100 μg of digest protein was spotted onto cellulose thin-layer sheets (20 by 20 cm) and electrophoresed in the first dimension at 600 V for 45 min in 10% pyridine–0.03% acetic acid, pH 4.5. The tryptic peptides were further separated by ascending chromatography at room temperature as described in Fig. 7. Positions of the hydrolysates were detected with 1% ninhydrin–0.1% cadmium acetate. To detect $^{32}$P-phosphopeptides the sheets were exposed to Kodak NS-54T X-ray film with a Dupont Lightning-Plus intensifying screen at −70°C for 2 days.

## RESULTS

Twenty two plaque-purified viral isolates were obtained from Ro*M*NPV wt-2. The viral DNAs from each were purified, restricted with *Eco*RI, and then electrophoresed in agarose gels. The restriction patterns of the 22 viral isolates, as well as the Ro*M*NPV wt-2 DNA, were the same as those shown for Ro*M*NPV R1 in Fig. 1. Three additional plaque-purified isolates, R9, AR7, and AR8, were obtained from Ro*M*NPV wt-1. The DNA restriction sites of one of these isolates (R9) have been physically mapped (8) and were the same as those of Ac*M*NPV E2 DNA with the exception of an additional *Eco*RI site between physical coordinants 59.2 and 70.2%. The other two Ro*M*NPV plaque-purified isolates, AR7 and AR8, will be shown to be recombinants of Ac*M*NPV and Ro*M*NPV. The restriction patterns of Ro*M*NPV wt-1 were indistinguishable from those of Ro*M*NPV R1 (data not shown).

**Ro*M*NPV R1 restriction endonuclease fragments.** Ro*M*NPV R1 DNA was cleaved with the restriction endonucleases *Eco*RI, *Xho*I, *Kpn*I, *Sac*I, *Bam*HI, and *Sma*I and electrophoresed in agarose gels. The fragment patterns are shown in Fig. 1 with individual fragments lettered sequentially by increased mobility. DNA bands which comigrate have been given a double-letter designation, e.g., *Eco*RI fragments IJ and MN. The molecular weights of Ro*M*NPV R1 restriction fragments (Table 1) were estimated by comparing their mobility in agarose gels with restriction fragments of Ac*M*NPV E2

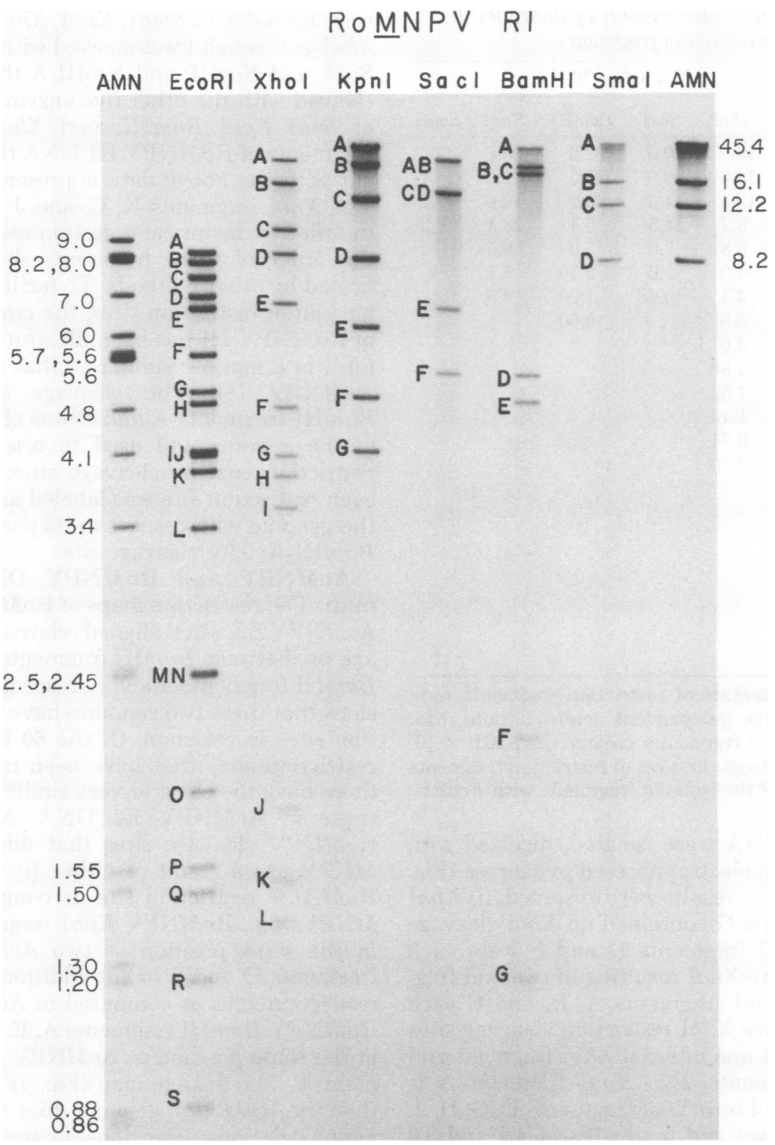


FIG. 1. *Autoradiogram of electrophoretically separated restriction fragments of RoMNPV R1 [$^{32}$P]DNA. The DNA was restricted with EcoRI, XhoI, KpnI, SacI, BamHI, and SmaI and then electrophoresed in 0.75% agarose. The EcoRI and SmaI fragments of AcMNPV E2 DNA were run in the first and last slots, respectively, as molecular weight standards. The size of each AcMNPV E2 fragment is labeled in megadaltons. Minor fragments in the EcoRI pattern were partially digested fragments and were not present in subsequent digests of RoMNPV R1 DNA.*

[$^{32}$P]DNA and λ [$^{32}$P]DNA (9). Ro*M*NPV R1 DNA has 22 *Eco*RI, 14 *Xho*I, 7 *Kpn*I, 7 *Sac*I, 8 *Bam*HI, and 4 *Sma*I restriction enzyme fragments. DNA bands of a molecular weight less than $0.3 \times 10^6$ would not have been detected. The sum of the molecular weights of the restriction bands for each enzyme was approximately $82.0 \times 10^6$.

**Restriction map of Ro*M*NPV R1 DNA.** The methods used to establish the order of restriction fragments have been presented in detail previously (8) in which the DNA restriction fragments of our Ac*M*NPV variants were mapped. As Ro*M*NPV and Ac*M*NPV DNA restriction maps are quite similar, only an example of the reciprocal double-digest data used to map Ro*M*NPV R1 DNA will be shown.

The *Kpn*I fragments A through G of Ro-

TABLE 1. *Molecular weights of RoMNPV R1 restriction fragments*

| Fragment | Mol wt[a] (× $10^6$) | | | | | |
|---|---|---|---|---|---|---|
| | *Eco*RI | *Xho*I | *Sac*I | *Bam*HI | *Kpn*I | *Sma*I |
| A | 8.7 | 19.5 | 20.0 | 32.5 | 26.6 | 45.4 |
| B | 8.0 | 14.8 | 20.0 | 18.7 | 18.5 | 16.1 |
| C | 7.8 | 10.4 | 14.5 | 16.2 | 12.6 | 12.2 |
| D | 7.0 | 8.7 | 14.5 | 5.4 | 8.4 | 8.2 |
| E | 6.9 | 6.8 | 7.0 | 5.0 | 6.5 | |
| F | 5.6 | 4.8 | 5.6 | 2.10 | 5.1 | |
| G | 5.0 | 4.1 | 0.68 | 1.20 | 4.0 | |
| H | 4.8 | 3.9 | | 0.60 | | |
| I | 4.1 | 3.6 | | | | |
| J | 4.1 | 1.80 | | | | |
| K | 4.0 | 1.52 | | | | |
| L | 3.4 | 1.40 | | | | |
| M | 2.50 | 0.74 | | | | |
| N | 2.50 | 0.70 | | | | |
| O | 1.90 | | | | | |
| P | 1.55 | | | | | |
| Q | 1.49 | | | | | |
| R | 1.20 | | | | | |
| S | 0.90 | | | | | |
| T | 0.78 | | | | | |
| U | 0.30 | | | | | |
| V | 0.30 | | | | | |

[a] Molecular weights of restriction fragments were the mean of three independent determinations. Molecular weights of fragments greater than 10.0 × $10^6$ were calculated from the sizes of restriction fragments after digestion of the isolated fragments with *Eco*RI.

*M*NPV [$^{32}$P]DNA were isolated, digested with *Xho*I, and then electrophoresed in agarose (Fig. 2). The following results were observed. (i) *Kpn*I fragments F and G contained no *Xho*I cleavage sites. (ii) *Kpn*I fragments D and E were each cleaved once by *Xho*I resulting in two end fragments. (iii) *Kpn*I fragments A, B, and C each had two or more *Xho*I restriction cleavage sites to give at least one internal *Xho*I fragment and two end fragments, e.g., *Kpn*I fragment A is cleaved by *Xho*I into *Xho*I fragments D, G, H, J, K, MN, and two end fragments of 4.2 and 3.0 megadaltons (Fig. 2).

A similar analysis was made with Ro*M*NPV R1 [$^{32}$P]DNA *Xho*I fragments A through I that had been digested with *Kpn*I (Fig. 2). By connecting the overlapping end fragments a partial order of *Kpn*I and *Xho*I fragments could be determined as A-(F,G)-D-B-E-C and B-A(L,MN)-E-I,F-C(D,K,G,J,H,MN), respectively. The following experiments established a restriction map for the Ro*M*NPV R1 genome. *Sma*I-A through D was cleaved with *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI; *Kpn*I-A through G was cleaved with *Sma*I, *Xho*I, *Sac*I, and *Eco*RI; *Bam*HI-A through G was cleaved with *Sma*I, *Kpn*I, and *Eco*RI; *Sac*I-A through F was cleaved with *Sma*I, *Kpn*I, *Xho*I, and *Eco*RI; *Xho*I-A through I was cleaved with *Sma*I, *Kpn*I, *Sac*I, and *Eco*RI; and *Eco*RI-A through Q was cleaved with the other five enzymes. The order of *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI fragments of Ro*M*NPV R1 DNA that is consistent with the above data is presented in Fig. 3. The *Xho*I fragments K, G, and J were difficult to order by reciprical digestion techniques, and the order of these fragments should be confirmed by other methods. To facilitate the comparison of restriction sites, the circular genome of Ro*M*NPV R1 has been illustrated as a linear form in a manner similar to that presented for Ac*M*NPV (8). The cleavage site between *Bam*HI fragments A and E was chosen as 0.0% of the genome and used to orient the other restriction enzyme cleavage sites. In addition, each restriction site was labeled as a percent of the genome with respect to its position and the *Bam*HI-A, 0.0% cleavage site.

**Ac*M*NPV and Ro*M*NPV DNA linkage map.** The restriction maps of Ro*M*NPV R1 and Ac*M*NPV E2, when aligned relative to the cleavage site between *Bam*HI fragments A and F and *Bam*HI fragments A and E, respectively (Fig. 4), show that these two genomes have many restriction sites in common. Of the 60 Ro*M*NPV R1 restriction sites that have been mapped, 35 of these have the same or very similar positions as those in Ac*M*NPV E2 DNA. Many of the Ro*M*NPV cleavage sites that differ from Ac*M*NPV are a result of either (i) the loss of a Ro*M*NPV restriction site as compared to Ac*M*NPV, e.g., Ro*M*NPV *Kpn*I fragment F maps in the same position as two Ac*M*NPV *Kpn*I fragments D and E or (ii) additional Ro*M*NPV restriction sites as compared to Ac*M*NPV, e.g., Ro*M*NPV *Bam*HI fragments A, E, and BC map in the same position as Ac*M*NPV *Bam*HI fragment A. The linkage map (Fig. 4) also suggests that the Ro*M*NPV genome does not have any major deletions, insertions, or inversions in its nucleotide sequence when compared to Ac*M*NPV.

**Recombinants of Ac*M*NPV and Ro*M*NPV.** The plaque-purified viruses AR7 and AR8 were isolated from Ro*M*NPV wt-1. The *Eco*RI restriction patterns of AR7 and AR8 DNAs (Fig. 5) when compared to Ac*M*NPV E2 and Ro*M*NPV R1, suggest that AR7 and AR8 are recombinants of Ac*M*NPV and Ro*M*NPV. Both AR7 and AR8 have *Eco*RI restriction fragments with mobilities in agarose gels which are either (i) the same as Ro*M*NPV R1 *Eco*RI fragments (ii) the same as Ac*M*NPV E2 *Eco*RI fragments or (iii) different from both R1 and E2 *Eco*RI fragments. Analysis of AR7 and AR8

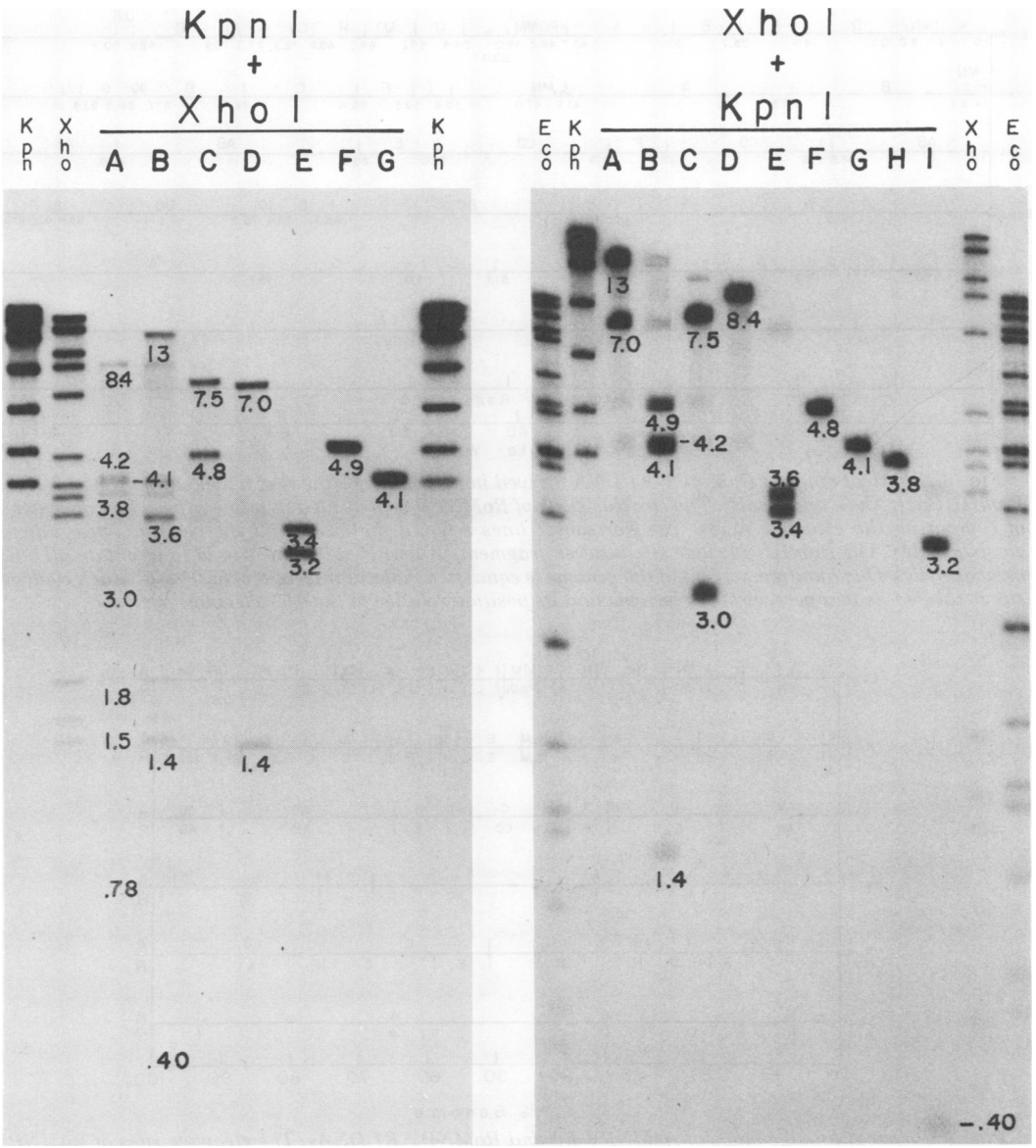


FIG. 2. *KpnI fragments A through G of RoMNPV R1 [$^{32}$P]DNA were purified from 0.6% Sea Plaque agarose gels, and isolated fragments were restricted with XhoI and electrophoresed in 0.75% agarose (KpnI + XhoI). XhoI fragments A through I of RoMNPV R1 [$^{32}$P]DNA were purified from 0.6% Sea Plaque agarose, restricted with KpnI, and electrophoresed in 0.75% agarose (XhoI + KpnI). The resulting DNA fragments were labeled in megadaltons. KpnI, XhoI, and EcoRI digests of RoMNPV R1 DNA were electrophoresed in adjacent slots to provide molecular weight standards.*

DNAs with *Bam*HI and *Xho*I (Fig. 5) gave similar results.

**Structural polypeptides of recombinants AR7 and AR8.** The viral structural polypeptides of the Ac*M*NPV and Ro*M*NPV recombinants, AR7 and AR8, were compared to the structural polypeptides of Ac*M*NPV variants S1, E2, and R9 and Ro*M*NPV R1 by polyacrylamide gel electrophoresis. Four Ac*M*NPV polypeptides with molecular weights of 90,000, 56,000, 40,000, and 37,000 are reproducibly distinct from four corresponding Ro*M*NPV polypeptides with molecular weights of 91,000, 55,000, 39,500, and 36,000 (Fig. 6). The recombinant, AR7, has the Ro*M*NPV major capsid polypeptides (molecular weight, 36,000) (10), the Ro*M*NPV polypeptides

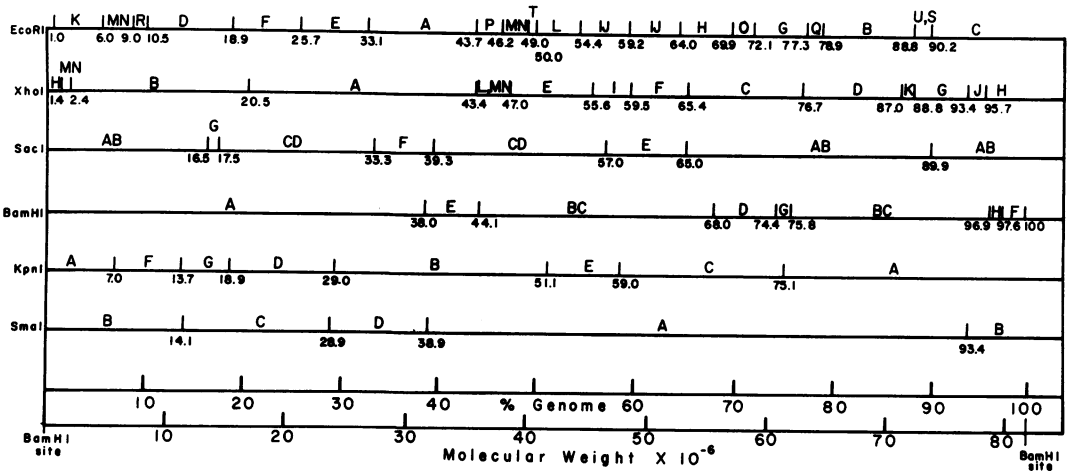


FIG. 3. *Physical maps of RoMNPV R1 DNA derived in this study for the restriction enzymes SmaI, KpnI, BamHI, SacI, XhoI, and EcoRI. The circular DNA of RoMNPV is presented in a linear form for convenience of comparing the cleavage maps. The horizontal lines refer to the DNA and the vertical lines indicate cleavage sites. The BamHI cleavage site between fragments A and F at 0.0% was used to orientate all other cleavage sites. One hundred percent of the genome is equal to a molecular weight of 82.0 × 10⁶. Each cleavage site is labeled as to a percent of the genome and its position relative to the 0.0% cleavage site.*

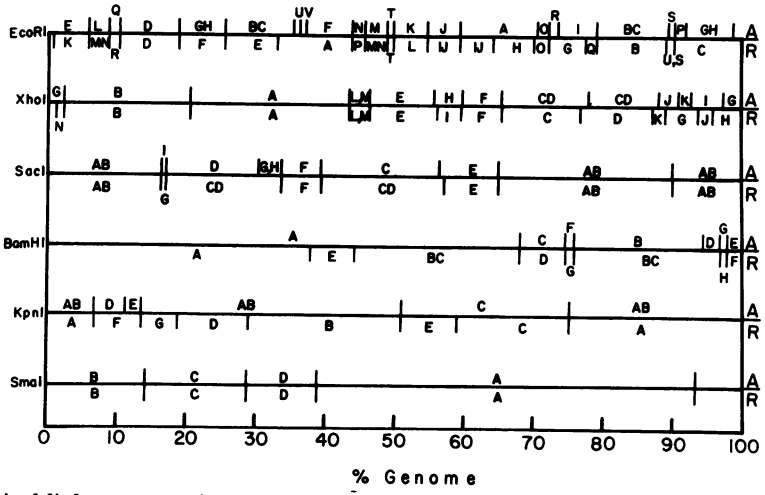


FIG. 4. *Physical linkage maps of AcMNPV E2 and RoMNPV R1 DNAs. The cleavage sites of RoMNPV R1 are illustrated as described in Fig. 3, and the AcMNPV E2 cleavage sites are as presented by Smith and Summers (8). The vertical lines above and below each horizontal are the cleavage sites of AcMNPV E2 and RoMNPV R1 DNAs, respectively.*

with molecular weights of 55,000 and 91,000, and the Ac*M*NPV structural polypeptides of 40,000 and 56,000 molecular weights. The AR8 recombinant also has the Ro*M*NPV capsid polypeptide of 36,000 molecular weight and the Ac*M*NPV polypeptides of 40,000, 56,000, and 90,000 molecular weights.

The tryptic peptides of $^{32}$P-labeled polyhedrin proteins of Ac*M*NPV E2, Ro*M*NPV R1, AR7, and AR8 were compared by two-dimensional analysis on thin-layer cellulose plates as described above. The tryptic peptide patterns of Ac*M*NPV E2 and Ro*M*NPV R1 are similar but have several distinct differences (Fig. 7). The tryptic peptide patterns of AR7 and AR8 polyhedrins are indistinguishable from that of Ac*M*NPV E2. An autoradiogram of Ac*M*NPV E2 and Ro*M*NPV R1 $^{32}$P-labeled polyhedrin tryptic peptides (Fig. 7) shows that both polyhedrins contain two major phosphopeptides and

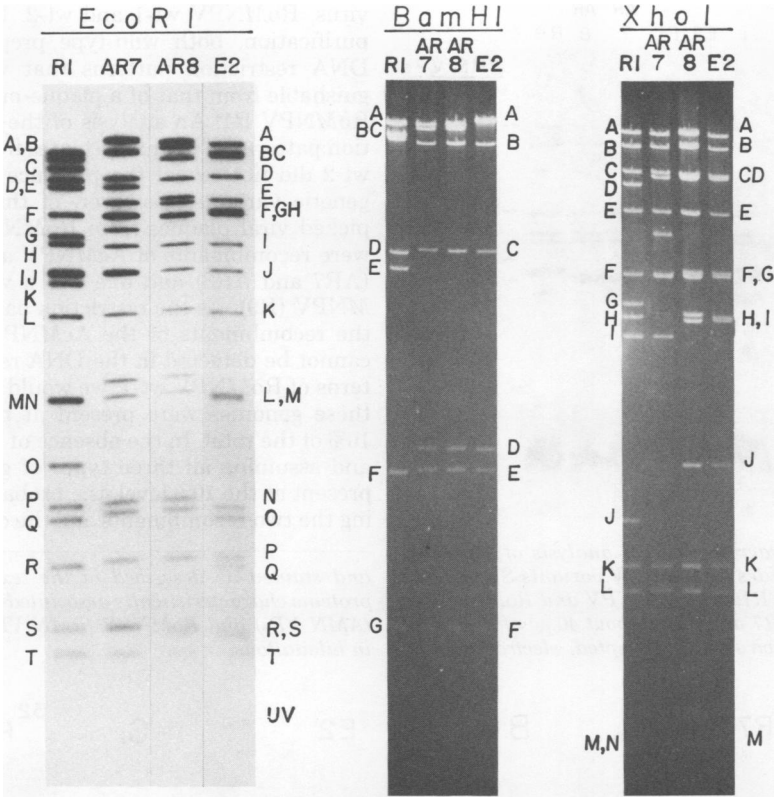


FIG. 5. *The EcoRI, BamHI, and XhoI restriction fragments of RoMNPV R1, AR7, AR8, and AcMNPV E2 DNAs were electrophoresed in 0.75% agarose. The RoMNPV R1 and AcMNPV E2 DNA restriction fragments are lettered as described in the text. The EcoRI digest is an autoradiogram of $^{32}$P-labeled viral DNAs and the BamHI and XhoI digests are the UV fluorescent bands of ethidium bromide-strained DNA.*

that one of the phosphopeptides of Ac*M*NPV E2 polyhedrin migrates differently when compared to the phosphopeptides of Ro*M*NPV R1 polyhedrin (Fig. 7). The phosphopeptides of AR7 and AR8 polyhedrin were the same as Ac*M*NPV E2 (data not shown). Thus, the AR7 and AR8 isolates have the Ac*M*NPV polyhedrin protein.

## DISCUSSION

In previous reports (2, 8, 9) wild isolates of Ac*M*NPV derived from infected insects have been shown to be a mixture of several genomic variants. In addition, the genomes of *Trichoplusia ni* (Tn*M*NPV) and *Galleria mellonella* (Gm*M*NPV) NPVs are also very similar to that of Ac*M*NPV (4, 9). Specifically, of the 60 restriction fragments mapped for Ac*M*NPV (9), Tn*M*NPV has 59 and Gm*M*NPV has 56 endonuclease fragments with similar mobilities in agarose as Ac*M*NPV fragments. That is, Tn*M*NPV and Gm*M*NPV both have greater than 90% of their restriction fragments in common with Ac*M*NPV. This degree of relatedness of the viral genomes is similar to the differences between Ac*M*NPV variants mapped in our laboratory (9). We propose that Gm*M*NPV and Tn*M*NPV should also be considered as variants of Ac*M*NPV. In contrast, only about 50% of the Ro*M*NPV restriction fragments had the same mobility in agarose gels as Ac*M*NPV. Therefore, Ro*M*NPV may not be as closely related to Ac*M*NPV as the Ac*M*NPV variants. Consistent with this observation are differences in several major structural polypeptides of Ro*M*NPV and Ac*M*NPV, including the polyhedrin gene. In comparison, differences in the major structural polypeptides among Ac*M*NPV variants or Tn*M*NPV were not observed (9). The major structural polypeptides of Gm*M*NPV are also very similar to those of Ac*M*NPV with the exception of the Ac*M*NPV protein of 40,000 molecular weight which is about 40,500 molecular weight in Gm*M*NPV (9).

In this study we obtained plaque-purified isolates of Ro*M*NPV from two sources of wild-type

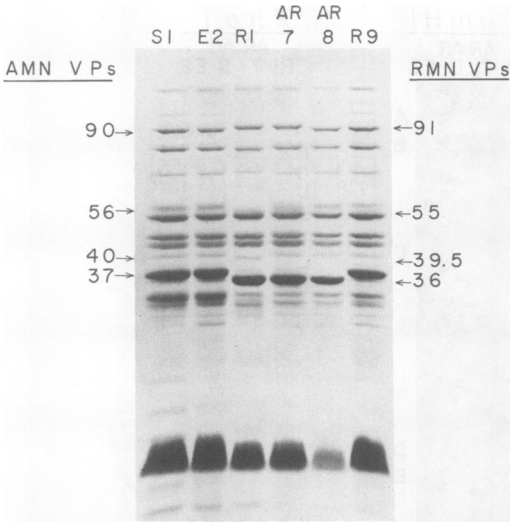


FIG. 6. *Polyacrylamide gel analysis of the structural polypeptides of AcMNPV variants S1, E2, and R9; RoMNPV R1; and AcMNPV and RoMNPV recombinants AR7 and AR8. About 40 μg of protein of each purified virus was disrupted, electrophoresed, and stained as described in the text. Those virus proteins characteristically associated with AcMNPV (AMN VPs) and RoMNPV (RMN VPs) are labeled in kilodaltons.*

virus, Ro*M*NPV wt-1 and wt-2. Before plaque purification, both wild-type preparations had DNA restriction patterns that were indistinguishable from that of a plaque-purified isolate, Ro*M*NPV R1. An analysis of the DNA restriction patterns of plaqued isolates from Ro*M*NPV wt-2 did not reveal the presence of Ro*M*NPV genetic variants. However, of three randomly picked viral plaques from Ro*M*NPV wt-1, two were recombinants of Ac*M*NPV and Ro*M*NPV (AR7 and AR8) and one was a variant of Ac*M*NPV (R9). As the restriction bands unique to the recombinants or the Ac*M*NPV variant R9 cannot be detected in the DNA restriction patterns of Ro*M*NPV wt-1, we would estimate that these genomes were present at no more than 10% of the total. In the absence of any selection, and assuming all three types of genomes were present at the 10% level, the probability of picking the two recombinants and the one Ac*M*NPV

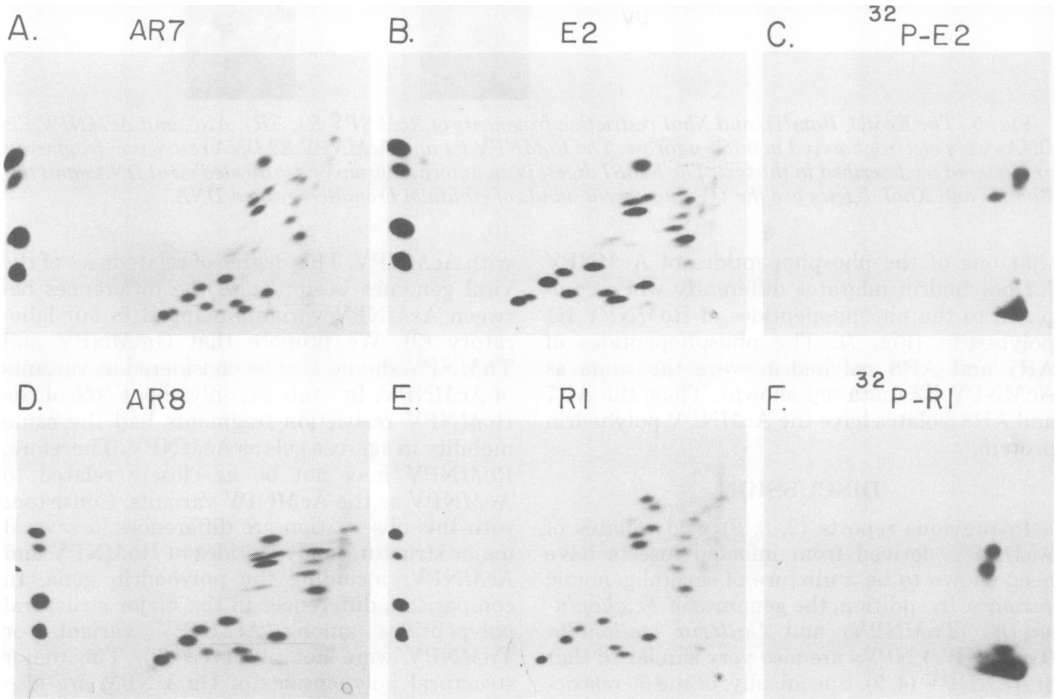


FIG. 7. *Two-dimensional analysis of the tryptic peptides of $^{32}$P-labeled polyhedrin protein from AcMNPV E2, RoMNPV R1, AR7, and AR8. The digests were spotted in the lower right, electrophoresed for 30 min at 750 V, and then chromatographed in butanol/pyridine/acetic acid/water (50:33:1:40). The ninhydrin-stained tryptic peptides are shown in A, B, D, and E, and the autoradiograms of AcMNPV E2 and RoMNPV R1 $^{32}$P-phosphopeptides are shown in C and F, respectively. A mixture of four amino acids was spotted on the left of each plate before being chromatographed.*

variant out of three randomly picked plaque would have been 1 in 1,000. One explanation for the occurrence of such an improbable event would be that genomes with certain Ac*M*NPV sequences are more efficient at replication as detected by plaque formation. Information in a subsequent report, where isolates from mixed infection of Ro*M*NPV and Ac*M*NPV will be analyzed, supports this hypothesis.

The loss of or acquisition of restriction sites as a result of mutation is the simplest interpretation for many of the differences observed between the restriction maps of Ro*M*NPV and Ac*M*NPV DNA. There is presently no evidence that these viruses have evolved through major deletions or by insertions of other viral or host genetic information. However, minor deletions or insertions in the genome of Ro*M*NPV relative to Ac*M*NPV could have gone undetected. Recombination may also have played a role in the evolution of Ro*M*NPV as the recombination frequency of Ro*M*NPV and Ac*M*NPV during mixed infection will be shown in a subsequent report to be high.

The segregation of Ac*M*NPV and Ro*M*NPV DNA restriction endonuclease cleavage sites as well as several viral structural proteins between the plaque-purified isolates AR7 and AR8 are convincing evidence that these are genetic recombinants. The recombination of baculovirus genes in nature and under controlled laboratory conditions may be important in the evolution of this group of viruses and in the development of our knowledge of the physical location and function of baculovirus genes.

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