JOURNAL OF VIROLOGY, Nov. 1984, p. 699–702
0022-538X/84/110699-04$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 52, No. 2

Conservation of Genome Organization in Two Multicapsid Nuclear Polyhedrosis Viruses†

DOUGLAS J. LEISY,* GEORGE F. ROHRMANN, AND G. S. BEAUDREAU

Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331

Received 7 May 1984/Accepted 20 July 1984

The genome of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata* was mapped by examining overlapping *Hin*dIII fragments from cosmid clones which had been constructed from partial *Hin*dIII digests of viral DNA. Five OpMNPV cosmid clones containing fragments encompassing the entire OpMNPV genome were hybridized to blots of DNA from the multicapsid nuclear polyhedrosis virus of *Autographa californica*. The hybridization pattern indicated that the genomes of these viruses are similarly organized.

Nuclear polyhedrosis viruses occur with either single or multiple nucleocapsids per envelope (SNPVs and MNPVs, respectively). In a previous study comparing two MNPVs and one SNPV by DNA-DNA hybridization under nonstringent conditions, we demonstrated that the MNPV of *Orgyia pseudotsugata* (OpMNPV) gave 24% duplex formation to the MNPV of *Autographa californica* (AcMNPV), and both MNPVs gave about 11% duplex formation to the SNPV of *Orgyia pseudotsugata* (9). Because DNA-DNA hybridization does not occur where there are nucleotide mismatches greater than one in three (2), the observed amount of duplex formation was suggestive of a high degree of evolutionary relatedness. The OpMNPV and AcMNPV genomes are very similar in size (6, 10), but their restriction profiles are quite dissimilar (3).

To arrive at a clearer understanding of the relatedness of the two MNPVs, we compared the organization of their genomes. The OpMNPV genome was mapped by cloning partially digested *Hin*dIII fragments into the cosmid pVK102. We then cross-hybridized labeled recombinant cosmid DNA to blots of the AcMNPV DNA restriction fragments. By identifying the AcMNPV fragments hybridized by each cosmid clone and locating these fragments on the AcMNPV map, we were able to determine that the genomes of these two viruses are predominantly colinear.

Construction of cosmid clones containing OpMNPV DNA was accomplished as follows: 0.2 μg of genomic viral DNA was partially digested with the restriction endonuclease *Hin*dIII and was mixed with 2 μg of *Hin*dIII-digested and phosphatase-treated pVK102 cosmid DNA (for construction of this vector, see reference 4) in a 10-μl ligase reaction containing 2 U of T4 DNA ligase and incubated at 16°C for 6 h.

Recombinant cosmids were packaged by an in vitro packaging system (Promega Biotec) and were selected by spreading infected Hb101 cells on plates containing LB agar (5) plus 12.5 μg of tetracycline per ml and incubating at 37°C overnight. Tetracycline-resistant colonies were then screened for susceptibility to kanamycin, and kanamycin-susceptible clones were given the designation pVKOM followed by an isolation number.

Recombinant clones were grown overnight in 10-ml cultures at 37°C in LB medium. Recombinant cosmid DNA was isolated by the alkaline lysis procedure as described by Maniatis et al. (5). The DNA was restricted with *Hin*dIII and electrophoresed on 0.8% agarose gels to determine the OpMNPV restriction fragments present in each clone. Table 1 summarizes the results from 37 clones. The order of *Hin*dIII fragments in the OpMNPV genome was readily determined from overlapping fragments between clones, except for the region containing fragments O and F and that containing fragments H and S. The order of fragments O and F was determined from the analysis of double digests with *Hin*dIII and other restriction enzymes. The order of fragments H and S remains undetermined. There is also a small *Hin*dIII restriction fragment of ca. 200 base pairs that is difficult to detect in ethidium bromide-stained gels but which can be detected by running end-labeled *Hin*dIII-cut total OpMNPV DNA on a polyacrylamide gel. This fragment was not mapped.

The positions of *Bam*HI, *Xba*I, and *Eco*RI restriction sites were determined by double digestion of selected cosmid clones with these enzymes and *Hin*dIII. The restriction site map is given in Fig. 1.

Five pVKOM clones (denoted in Table 1) were selected which together contain *Hin*dIII fragments spanning the entire genomic map. DNAs from these clones and from a nonrecombinant pVK102 plasmid were labeled by nick translation and hybridized to blots containing *Hin*dIII-digested OpMNPV DNA and *Hin*dIII and *Pst*I digests of AcMNPV DNA (for sources of DNA, see reference 9). Figure 2 shows the restriction profiles of these digests in an ethidium bromide-stained gel. Initial experiments were done to determine stringency conditions which allow detection of cross-hybridization to regions of limited homology while minimizing totally nonspecific hybridization. We found that hybridizations conducted at 42°C in a standard hybridization buffer containing 30% formamide (for methods, see legend to Fig. 3) were best (data not shown).

A control blot hybridized with nonrecombinant pVK102 DNA showed weak hybridization to a number of bands (Fig. 3). The weak hybridization to the high-molecular-weight bands is thought to be mainly due to nonspecific hybridization, which is detectable only because of the large mass of DNA present in these bands. However, hybridization to the lower-molecular-weight OpMNPV B and G, AcMNPV *Hin*dIII bands T or U or both, and AcMNPV *Pst*I bands M or N or both suggests that there is at least a weak homology between DNA sequences in these bands and DNA sequences in the control probe.

As expected, the cloned DNAs specifically hybridized to the *Hin*dIII OpMNPV fragments contained in each clone

* Corresponding author.
† Technical Paper no. 7176 from the Oregon State Experimental Station.

TABLE 1. *Hin*dIII fragment order of the OpMNPV genome and of pVKOM cosmid clones

Isolate no.[a]	*Hin*dIII fragments	Insert size (kb)
64	A	21.0
49, 62	AT	22.5
27	ATN	26.4
1*, 50	N(HS)IRK	23.0
13, 24, 46	IRKC	26.0
12	IRKCL	29.9
45	RKC	21.3
2, 61	KCL	21.9
20, 37	CL	17.8
54*	CLD	29.3
34	CLDU	30.3
41	DUB	28.6
43	UB	17.1
58*	UBP	20.8
67	UBPJ	25.6
17, 55	BP	19.7
14, 15	BPJ	24.1
9, 35, 60	BPJG	29.3
63	PJGOF	23.6
39*	JGOFE	28.3
26	GOFE	23.9
5	GOFEM	27.8
22, 52, 57	OFEMQ	26.1
48	MQA	28.5
47*	MQAT	30.0

[a] Clones used in the hybridization studies are marked with an *.

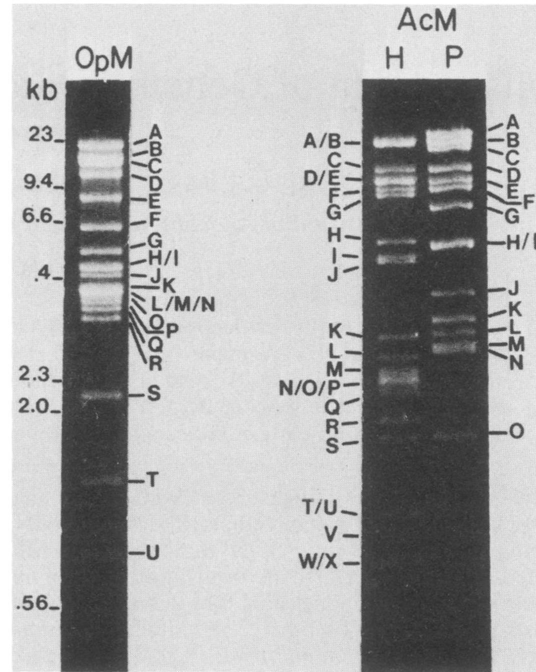

FIG. 2. Restriction digests of OpMNPV and AcMNPV. OpMNPV (OpM) DNA was digested with *Hin*dIII. AcMNPV (AcM) was digested with *Hin*dIII (H) and *Pst*I (P). Each digest (1 μg) was electrophoresed on a 0.8% agarose gel prepared in Tris-acetate-EDTA buffer containing 0.5 μg of ethidium bromide per ml.

(Fig. 3). Some clones also showed weaker hybridization to additional OpMNPV bands not contained within the limits of the clone. These findings suggest that the OpMNPV genome may have repeated sequences similar to those found in the AcMNPV genome (1). These repeated sequences are likely present within OpMNPV *Hin*dIII fragments A and B or C or both (as clearly seen in the hybridization to these bands with the pVKOM-39 probe, Fig. 3A), E, and F (as seen in the hybridization patterns when pVKOM-47, pVKOM-54, and pVKOM58 are used as probes, Fig. 3A). Figure 3 also shows the hybridization patterns of the selected pVKOM clones to *Hin*dIII and *Pst*I digests of AcMNPV. Table 2 summarizes the hybridization results. In some cases, hybridization to a particular band in a set of closely migrating bands could be discerned by analyzing the hybridization pattern in the other digest and referring to the restriction site map. For example, hybridization to *Hin*dIII fragment I by pVKOM-54 could be distinguished from hybridization to *Hin*dIII fragment J because *Pst*I fragment L was hybridized and *Pst*I fragment I was not (see the AcMNPV map in Fig. 4).

Some of the AcMNPV fragments were not hybridized by any of the pVKOM probes (Table 2). For instance, pVKOM-1 did not hybridize to the region of the genome containing AcMNPV *Hin*dIII fragments X and J but hybridized strongly to fragments to either side of this region. The regions containing AcMNPV *Hin*dIII fragments K and S also

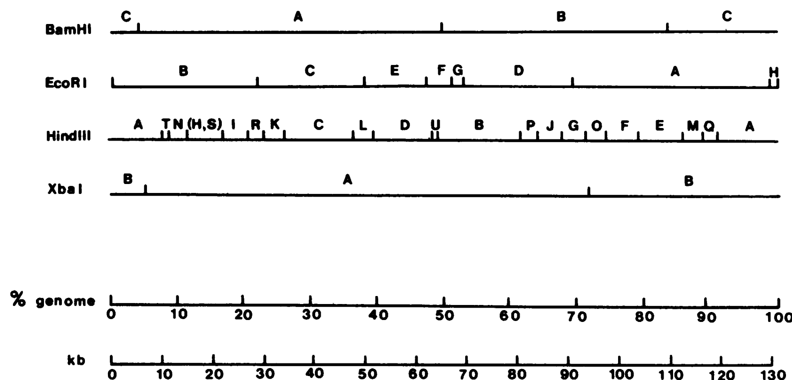

FIG. 1. Restriction site map of OpMNPV. The positions of the *Hin*dIII restriction sites were determined by the analysis of overlapping OpMNPV sites between different pVKOM cosmid clones. *Bam*HI, *Eco*RI, and *Xba*I sites were determined by the gel analysis of single- and double-enzyme digests of selected pVKOM cosmids with these enzymes and *Hin*dIII.

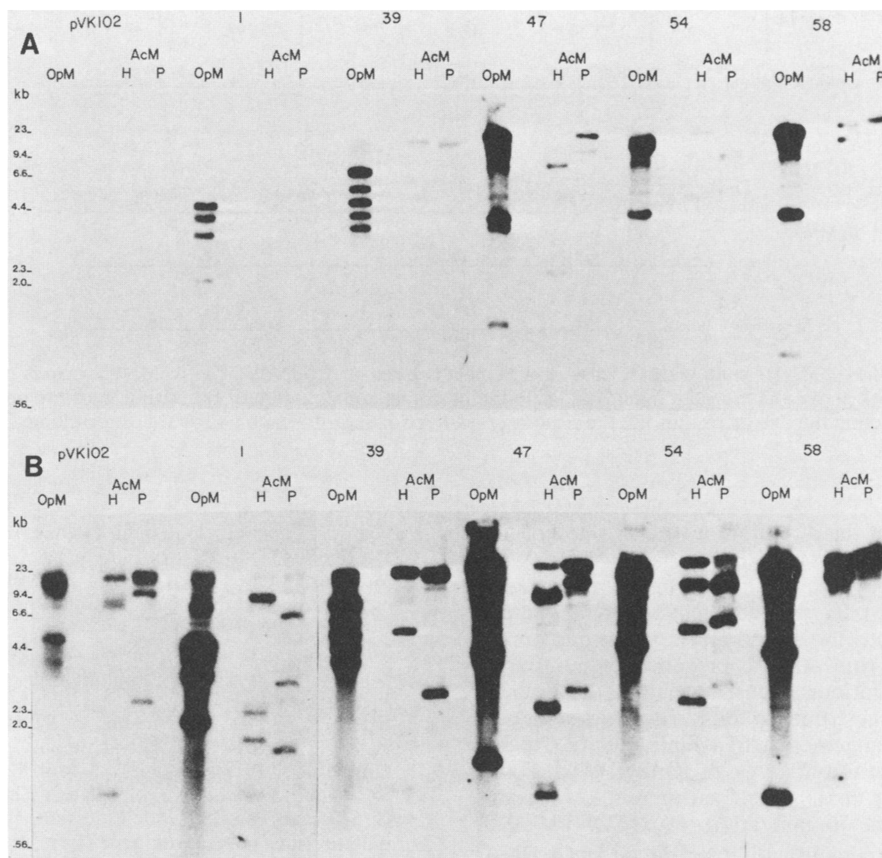

FIG. 3. Hybridization of selected pVKOM cosmid clones to *Hin*dIII digests of OpMNPV DNA and AcMNPV and *Pst*I digests of AcMNPV DNA. Gels prepared as described in the legend to Fig. 2 were blotted onto nitrocellulose paper by the bidirectional blotting method of Smith and Summers (12). DNA from selected pVKOM cosmid clones and a pVK102 control was labeled by nick translation with [^{32}P]dATP (7). Nick-translated DNA from each clone was hybridized (ca. 3×10^6 cpm per nitrocellulose blot) for 22 h at 42°C in a solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10 mM EDTA, and 30% formamide. Blots were washed at room temperature for 5 min, transferred to a fresh tray, and then washed for 15 min in solutions containing 2× SSC and 0.1% sodium dodecyl sulfate. This was followed by washings at room temperature for 2 h and then for 15 min in solutions containing 0.1× SSC and 0.1% sodium dodecyl sulfate. (A) Blots were exposed to X-Omat-R film for 12 h. (B) Blots were exposed for 5 days (pVK102, pVKOM-1) or 3 days (pVKOM-39, pVKOM-47, pVKOM-54, pVKOM-58). Exposure times were varied to compensate for unequal transfer of DNA during the bidirectional blotting procedure.

showed no hybridization to any of our probes. Interestingly, these three regions of the AcMNPV genome correspond very closely to regions which are also not hybridized by labeled *Anticarsa gemmatalis* MNPV DNA (13). This suggests either that these regions may vary extensively among a number of related MNPV types, possibly because they are under less functional constraint than the majority of the genome, or that they may have arisen independently in the AcMNPV line at a time later than the divergence of these other two MNPV types, possibly from a nonviral origin. Smith and Summers (13) also identified an additional nonhybridizing region at *Eco*RI-R, and we have identified one at *Pst*I-L. These may also be hypervariable regions or regions of independent origin in the AcMNPV line. We expect that even more such regions could be found by hybridizing the labeled probes to blots of a large number of digests with different restriction enzymes.

Figure 4 shows the alignment of the restriction site maps of AcMNPV and OpMNPV. We find that within the limits of our study, the cross-hybridizing regions of the two viruses are arranged predominantly in a colinear fashion. One exception to a strict colinear arrangement is found in the region containing AcMNPV *Hin*dIII fragment L and *Pst*I fragment J, which hybridizes weakly with both pVKOM-1 and pVKOM-54. This may result from cross-hybridization of repetitive sequences in OpMNPV with the repetitive sequence reported by Cochran and Faulkner (1) to lie within AcMNPV fragment L, or it may be an indication of a translocation from this region occurring in the OpMNPV since the time of evolutionary divergence with AcMNPV. It

TABLE 2. AcMNPV fragments hybridized by pVKOM cosmid clones

Cosmid	AcMNPV fragments hybridized[a]	
	*Hin*dIII	*Pst*I
pVKOM-1	D, L,[b] M, R	G, J,[b] K, O
pVKOM-39	B, H, W	B, C, M
pVKOM-47	G, F, (N, Q, P),[c] V	B, D, N
pVKOM-54	A, E, I, L,[b] O	E, F, H, J[b]
pVKOM-58	A, C	A

[a] *Hin*dIII fragments T or U or both and *Pst*I fragments M or N or both were hybridized weakly by all clones tested, including a pVK102 control. *Hin*dIII fragments J, K, S, and X and *Pst*I fragments I and L were hybridized.

[b] Hybridization barely detectable in the longer exposure (see Fig. 3).

[c] Bands not resolvable in the autoradiographs.

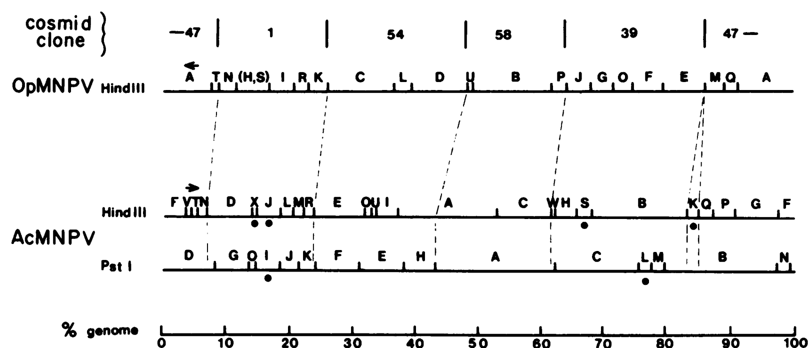

FIG. 4. Alignment of the OpMNPV map with the cross-hybridizing regions of AcMNPV. The AcMNPV map is taken from that of Vlak and Smith (14). The arrows above the restriction maps indicate the positions and direction of transcription of the polyhedrin gene for each virus. Dashed lines connecting the two maps indicate the regions cross-hybridizing with each pVKOM cosmid clone. Dots indicate AcMNPV fragments not hybridized.

is interesting to find that the direction of transcription of the polyhedrin genes in these two viruses is opposite with respect to the aligned maps (8, 11), indicating the presence of an inversion. This is the only gene which has been characterized well enough in both viruses to allow a comparison at this level. We cannot rule out the presence of additional inversions and translocations occurring within the boundaries of our cosmid clones, but we found no other evidence of major genomic rearrangements. In a similar set of experiments, blots of restriction digests of OpSNPV did not produce enough specific cross-hybridization to allow a comparison of the genome organization of OpMNPV and OpSNPV. This is in agreement with the reduced DNA-DNA hybridization between the OpSNPV and the two MNPVs (9).

ACKNOWLEDGMENTS

We thank Michael Nesson and John Armstrong for their careful review of this manuscript.

This work was supported in part by Public Health Service grant ES0219 from the National Institutes of Health.

LITERATURE CITED

1. **Cochran, M. A., and P. Faulkner.** 1983. Location of homologous DNA sequences interspersed at five regions in the baculovirus AcMNPV genome. J. Virol. **45**:961–970.
2. **Howley, P. M., M. A. Israel, M. Law, and M. A. Martin.** 1979. A rapid method for detecting and mapping homology between heterologous DNAs. J. Biol. Chem. **254**:4876–4883.
3. **Jewell, J., and L. K. Miller.** 1979. DNA sequence homology relationships among six Lepidopteran nuclear polyhedrosis viruses. J. Gen. Virol. **48**:161–175.
4. **Knauf, V. C., and E. W. Nester.** 1982. Wide host range cloning vectors: a cosmid clone bank of an *Agrobacterium* Ti plasmid. Plasmid **8**:45–54.
5. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Miller, L. K., and K. P. Dawes.** 1979. Physical map of the DNA genome of *Autographa californica* nuclear polyhedrosis virus. J. Virol. **29**:1044–1055.
7. **Rigby, P. W., M. Diechmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **133**:237–251.
8. **Rohrmann, G. F., D. J. Leisy, K.-C. Chow, G. D. Pearson, and G. S. Beaudreau.** 1982. Identification, cloning and R-loop mapping of the polyhedrin gene from the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*. Virology **121**:51–60.
9. **Rohrmann, G. F., M. E. Martignoni, and G. S. Beaudreau.** 1982. DNA sequence homology between *Autographa californica* and *Orgyia pseudotsugata* nuclear polyhedrosis viruses. J. Gen. Virol. **62**:137–143.
10. **Schafer, M. P., G. Rohrmann, U. Heine, and G. S. Beaudreau.** 1979. DNA from two *Orgyia pseudotsugata* baculoviruses: molecular weight determination by means of electron microscopy and restriction endonuclease analysis. Virology **95**:176–184.
11. **Smith, G. E., J. M. Vlak, and M. D. Summers.** 1983. Physical analysis of *Autographa californica* nuclear polyhedrosis virus transcripts for polyhedrin and 10,000-molecular-weight protein. J. Virol. **45**:215–225.
12. **Smith, G. E., and M. D. Summers.** 1980. Restriction map of *Rachiplusia ou-Autographa californica* baculovirus recombinants. J. Virol. **33**:311–319.
13. **Smith, G. E., and M. D. Summers.** 1982. DNA homology among subgroup A, B and C baculoviruses. Virology **123**:393–406.
14. **Vlak, J. M., and G. E. Smith.** 1982. Orientation of the genome of *Autographa californica* nuclear polyhedrosis virus: a proposal. J. Virol. **41**:1118–1121.