Molecular Cloning and Physical Mapping of Restriction Endonuclease Fragments of *Autographa californica* Nuclear Polyhedrosis Virus DNA

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A restriction fragment library containing *Autographa californica* nuclear polyhedrosis virus (AcNPV) DNA was constructed by using the pBR322 plasmid as a vector. The library, which is representative of more than 95% of the viral genome, consists of 2 of the 7 *Bam*HI fragments, 12 of the 24 *Hind*III fragments, and 23 of the 24 *Eco*RI fragments. The cloned fragments were characterized and used to generate physical maps of the genome by hybridizing nick-translated recombinant plasmid to Southern blots of AcNPV DNA digested with *Sma*I, *Bam*HI, *Xho*I, *Pst*I, *Hind*III, and *Eco*RI restriction endonucleases. This information was used to define our strain of AcNPV (HR3) with respect to other strains for which physical maps have been previously published. The hybridization data also indicate that reiteration of DNA sequences occurs at the *Hind*III-L and -Q regions of the genome.

The nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica* (AcNPV), is a prototype for molecular studies of insect baculoviruses. The replicative cycle of the virus in permissive insect cell lines is complex but can be divided into two phases (4, 6). First, singly enveloped nucleocapsids, termed nonoccluded viruses (NOV), are budded from the cell membrane during the early stages of infection. Second, later in the infectious cycle, production of NOV is curtailed and occlusion bodies (OB), which consist of enveloped bundles of nucleocapsids lying within a paracrystalline protein matrix, form in the cell nuclei. The genome of AcNPV consists of a double-stranded, superhelical, circular DNA molecule of approximately 128 kilobase pairs which has been physically mapped for several restriction enzyme sites (9, 12, 17). Wild isolates of AcNPV derived from insects can be separated by plaque purification into variant strains having minor alterations in DNA restriction patterns (8, 11, 15).

As a prelude to generating a transcription map of the AcNPV genome, we cloned almost the entire genome of AcNPV as *Bam*HI, *Eco*RI, and *Hind*III fragments in the plasmid pBR322. The recombinant plasmids were characterized and authenticated by restriction enzyme digestion and by hybridization to restricted AcNPV DNA. These data also served to identify our wild-type strain (HR3) as being quite similar to the E2 strain of Smith and Summers (12) and the L1 strain of Miller and Dawes (9) by confirming their physical maps for most of the *Sma*I,

*Bam*HI, *Xho*I, and *Eco*RI restriction fragments. Furthermore, complete physical maps for the restriction enzymes *Hind*III and *Pst*I are presented.

MATERIALS AND METHODS

Purification of virus and viral DNA. A plaque-purified MP (many OB/cell) strain of AcNPV, designated here as HR3, which grew at both 28 and 33°C (1) was propagated on monolayers of *Spodoptera frugiperda* cells at 28°C (16). Cells were infected at a multiplicity of 5 to 10 PFU per cell, and extracellular NOV and OB were isolated as described by Dobos and Cochran (3). Viral DNA was isolated from gradient-purified NOV as described by Tjia et al. (15). Briefly, the NOV suspension (2 to 5 mg of protein/ml in 0.1 M Tris-0.01 M EDTA, pH 7.5), was incubated at 37°C for 1 h in 1% (wt/wt) sodium dodecyl sulfate (SDS), 1% (wt/wt) sodium lauryl sarcosinate, and 500 µg of proteinase K per ml (Merck & Co., Inc., Rahway, N.J.). Subsequently, pronase B was added to 500 µg/ml, and incubation was continued for 1 h. The solution was extracted three times with buffer-saturated phenol and once with chloroform-isoamyl alcohol (24:1). The aqueous phase was dialyzed extensively against 0.1× SSC (1× SSC is 0.015 M sodium citrate and 0.15 M sodium chloride, pH 7.5). To isolate DNA from OB, gradient-purified OB (5 to 10 mg of protein/ml) were suspended in 0.1 M Na₂CO₃, 0.17 M NaCl, and 0.01 M EDTA, pH 10.9, and incubated at 37°C for 15 min to liberate the occluded virions. DNA was purified from this solution as described above.

Purification of plasmid DNA. The bacterium HB101 (rk⁻ mk⁻; obtained from B. N. White, Department of Biology, Queen's University), containing the plasmid pBR322 or recombinant plasmids, was grown in either 5 to 10 ml (miniprep) or 1 liter of Luria broth contain-

ing 20 μ g of the antibiotics tetracycline and ampicillin per ml, alone or together. Cell suspensions were grown at 37°C in a shaker bath, and the plasmids were amplified during logarithmic growth by the addition of chloramphenicol (200 μ g/ml) (2). The cells were harvested, and a cleared lysate was prepared as described by Guerry et al. (7) with some modifications. Briefly, the pellets from miniprep and 1-liter cultures were suspended in 0.5 and 20 ml, respectively, of cold STEN buffer (25% sucrose, 50 mM Tris, pH 8.0, 20 mM EDTA, and 100 mM NaCl). After addition of fresh lysozyme (Sigma Chemical Co., St. Louis, Mo.) to 1.5 mg/ml, the suspension was brought to 1% (wt/vol) SDS and 1 M NaCl. Lysates from 1-liter cultures were incubated on ice overnight and then centrifuged at 17,000 $\times$ g for 45 min at 4°C. Minipreps were left on ice for 45 min and centrifuged in a Beckman microfuge (15,000 $\times$ g) at 4°C. The supernatant from these centrifugations was designated the cleared lysate.

To purify candidate recombinant plasmids from minipreps, 0.5-ml volumes of cleared lysates were extracted sequentially with water-saturated phenol and with chloroform in Eppendorf tubes. The DNA was then precipitated with 2 volumes of ethanol at -70°C for 30 min, and the precipitate (5 to 10 μ g of plasmid DNA) was dissolved in 50 μ l of TE buffer (10 mM Tris-1 mM EDTA, pH 7.5).

Superhelical plasmid DNA was purified from the cleared lysates of 1-liter cultures, using the acid-phenol extraction method of Zasloff et al. (19) as described by Gordon et al. (5).

Construction of recombinant plasmids. In three separate experiments, AcNPV and pBR322 DNAs were each digested to completion with either *Bam*HI, *Hind*III, or *Eco*RI. Each sample was then chloroform extracted, ethanol precipitated, dried, resuspended in TE buffer, and heated to 65°C for 5 min. A 50- μ l ligation reaction mixture contained 0.5- μ g of digested pBR322, 1.0 to 3.5 μ g of digested viral DNA, and 0.1 U of T4 DNA ligase (Bethesda Research Laboratories, Rockville, Md.) in 50 mM Tris-hydrochloride, pH 7.6, 10 mM MgCl₂, 0.1 mM EDTA, 1 mM dithiothreitol, and 1 mM ATP (J. Hassel, personal communication). The ligation reaction occurred during an 18-h incubation at 12°C for *Bam*HI and *Hind*III ligations and at 0°C for *Eco*RI ligations.

Competent HB101 cells were prepared as follows (B. White, personal communication). Cells were grown in 100 ml of Luria broth to an absorbancy at 260 nm of 0.6, chilled on ice, and centrifuged at 2,500 $\times$ g for 5 min at 4°C. The pellet was suspended in 25 ml of cold 10 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES), pH 6.8-10 mM RbCl, pelleted as before, and suspended in 10 ml of cold 10 mM PIPES, pH 6.8-10 mM RbCl-75 mM CaCl₂. After incubation at 0°C for 30 min, the cells were pelleted again and gently suspended in 5 ml of ice-cold 10 mM PIPES, pH 6.8-10 mM RbCl-75 mM CaCl₂-15% glycerol. The cells were divided into equal portions, frozen in a dry ice-isopropanol bath, and stored at -70°C. Competent cells (200 μ l) were transformed by addition of the ligation mixture (100 μ l) containing 100 mM NaCl. The mixture was maintained on ice for 20 min, incubated at 25°C for 10 min, and, after supplementation with 1 ml of growth medium, incubated for 40 min at 37°C. Cells were then plated on medium containing the appropriate antibiotics. Under these conditions, the efficiency

of transformation was typically about 2×10^6 transformants/ μ g of uncut pBR322.

Ampicillin-resistant bacteria were selected and screened for susceptibility to tetracycline in the *Bam*HI and *Hind*III ligation experiments. These Ap^r Tc^s clones and the Ap^r Tc^s clones from the *Eco*RI ligation experiments were screened by colony hybridization (14), using nick-translated AcNPV DNA as the probe.

Restriction endonucleases, gel electrophoresis, blotting, hybridization, and nick translation. The *Sma*I, *Bam*HI, *Xho*I, *Pst*I, *Hind*III, and *Eco*RI restriction endonucleases were purchased from either Bethesda Research Laboratories or Boehringer Mannheim (Dorval, Quebec). The conditions for restriction of DNA were as described in the protocols supplied with the restriction enzyme.

Restriction fragments of AcNPV DNA and plasmid DNA were separated by electrophoresis in 0.8% agarose gels in 100 mM Tris-hydrochloride, pH 9.0, 7.7 mM H₃BO₃, 2.5 mM EDTA, and 0.5 μ g of ethidium bromide per ml at 1.5 V/cm for 16 h on horizontal slab gels. DNA fragments used as standards for size determinations were the *Hind*III fragments of lambda DNA (Bethesda Research Laboratories), the *Hae*III fragments of ϕ OX174 (Bethesda Research Laboratories), and the *Hpa*II fragments of pBR322.

After electrophoresis, the restricted DNA was transferred onto nitrocellulose filters, using the Southern technique (13) as previously described (15). Viral sequences were detected by hybridization of ³²P-labeled AcNPV DNA or recombinant plasmid DNA to the Southern blots. The conditions of hybridization were as described by Wahl et al. (18). Occasionally hybridizations were performed under more stringent conditions at 65°C in 4 $\times$ SSC, 0.1% SDS, 50 μ g of sonicated and denatured salmon sperm DNA per ml, plus Denhardt solution (Ficoll, polyvinylpyrrolidone, and bovine serum albumin; 2% each). Under either of these conditions, the amount of DNA used in the gel was 0.025 μ g per well. DNA was labeled with ³²P by nick translation, using the method of Rigby et al. (10) as described by Tjia et al. (15).

RESULTS

Restriction endonuclease fragment patterns of HR3 AcNPV DNA. The fragment patterns of *Sma*I, *Bam*HI, *Xho*I, *Pst*I, *Hind*III, and *Eco*RI are shown in Fig. 1. There were no detectable differences between the restriction patterns of AcNPV DNA purified from OB or NOV (data not shown). The DNA used in these studies was from both sources. Individual fragments were assigned an alphabetical designation on the basis of size: the largest fragment for each enzyme was referred to as A, the second largest was referred to as B, etc. The sizes of the AcNPV DNA fragments were estimated by comparing their mobilities in agarose gels with standard fragments of known numbers of bases (Table 1). The sum of the sizes of the fragments generated by the enzymes listed in Table 1 indicated that the AcNPV genome contained about 128 kilobase pairs. The *Xho*I-N and *Eco*RI-X fragments

FIG. 1. Agarose gel electrophoresis of AcNPV DNA after digestion with *Sma*I, *Bam*HI, *Xho*I, *Pst*I, *Hind*III, and *Eco*RI restriction endonucleases. Conditions of electrophoresis were those described in Materials and Methods.

are in addition to those reported by Smith and Summers (12), and the *Hind*III-V, -W, and -X fragments are in addition to those reported by Miller and Dawes (9).

Differences between our HR3 strain, the E2 variant of Smith and Summers (12), and the L1 strain of Miller and Dawes (9) were evident upon *Hind*III digestion (Fig. 2). Digestion with *Sma*I, *Bam*HI, *Xho*I, *Eco*RI, and *Pst*I showed identical patterns among the three strains, except that the *Pst*I-J fragments of E2 and L1 were 200 base pairs (bp) smaller than that of HR3. The sole difference between HR3 and E2 was that the *Hind*III-L' fragment of E2 was about 200 bp smaller than that of HR3 (Fig. 2). Differences between HR3 and L1 included: an extra *Hind*III site in the *Hind*III-A fragment generating the *Hind*III-B' and -H' fragments of L1; and an L1 *Hind*III-L' that was 200 bp smaller than the HR3 *Hind*III-L fragment.

Construction of recombinant plasmid library. To obtain a collection of plasmids which contained sequences from the viral genome, total restriction digests of AcNPV DNA were ligated to pBR322 and used to transform *Escherichia coli* strain HB101. DNA from three sets of digestions was used: (i) AcNPV and pBR322 DNAs cleaved with *Bam*HI; (ii) AcNPV and pBR322 DNAs cleaved with *Hind*III; and (iii) AcNPV and pBR322 DNAs cleaved with *Eco*RI. After transformation of the *E. coli* strain HB101,

TABLE 1. Sizes of AcNPV DNA restriction fragments and enumeration of clones

Frag- ment	Size ^a (kilobase pairs)							
	<i>Eco</i> RI	Cloned	<i>Hind</i> III	Cloned	<i>Pst</i> I	<i>Xho</i> I	<i>Bam</i> HI	Cloned
A	14.2	+ ^b	21.5		25.7	29.2	86.5	
B	13.3	+	20.0		21.6	23.2	23.3	
C	12.2	+	11.1		17.1	14.4	8.50	
D	10.4	+	9.95		11.5	14.4	3.45	
E	9.00	+	9.95	+	10.4	10.4	3.33	+
F	8.78	+	8.40		8.44	7.65	1.92	+
G	8.70	+	8.15	+	6.91	7.58	0.96	
H	8.70	+	5.60	+	5.12	6.40		
I	7.55	+	5.02		4.86	6.12		
J	6.66		4.74		3.45	3.29		
K	5.38	+	2.75	+	3.00	2.26		
L	3.78	+	2.62	+	2.90	2.14		
M	3.65	+	2.30	+	2.81	1.13		
N	2.38	+	2.23	+	2.62	0.35		
O	2.25	+	2.23		1.66			
P	1.98	+	2.10					
Q	1.93	+	2.04	+				
R	1.41	+	1.79	+				
S	1.38	+	1.65	+				
T	1.28	+	1.05					
U	0.942	+	1.02	+				
V	0.937	+	0.926					
W	0.515	+	0.777	+				
X	0.510	+	0.773					
Total	127.81		128.66		128.07	128.52	127.96	127.90

^a The size of each restriction fragment was determined as the average of at least 10 independent determinations, using *Hind*III fragments of lambda DNA, *Hae*III fragments of ϕ X174 DNA, and *Hpa*II fragments of pBR322 as standards. Sizes of fragments greater than 15 kilobases were calculated from the sizes of restriction fragments generated after *Eco*RI digestion.

^b +, Cloned into pBR322.

FIG. 2. Agarose gel electrophoresis of HR3, E2 (11), and L1 (9) AcNPV DNA digested with *Hind*III restriction endonuclease. Fragments which differed in electrophoretic mobility with respect to HR3 are (i) L' of E2 and L1 and (ii) B' and H' of L1.

bacterial clones were screened for the appropriate antibiotic phenotype, i.e., Ap^r Tc^r phenotype for *Bam*HI ligations and Ap^r Tc^r phenotype for *Hind*III ligations. Clones from the *Eco*RI ligation experiments were first selected for the Ap^r Tc^r phenotype and then screened along with the clones from the other experiments by colony hybridization, using nick-translated ³²P-labeled AcNPV DNA as the probe. Clones which gave a positive hybridization signal were then amplified, and recombinant plasmid DNA was purified. The DNA from these preparations was digested with the appropriate enzyme in order to cut out the inserted viral DNA and analyzed by gel electrophoresis along with similarly digested AcNPV DNA. Analysis of the recombinant plasmids showed that all of the 24 *Eco*RI fragments except J, 12 of the 24 *Hind*III fragments, and 2 of the 7 *Bam*HI fragments were cloned (Table 1). Figure 3 shows examples of recombinant plasmids containing AcNPV DNA *Eco*RI fragments. Some of the recombinant plasmids contained more than one inserted viral fragment, e.g., the plasmid pAcEcoCX18 contained the *Eco*RI fragments C and X.

Confirmation of AcNPV DNA sequences in recombinant plasmids. AcNPV DNA sequences in recombinant plasmids were identified and characterized by the following techniques: colony hybridization; electrophoretic mobility of cloned fragments with respect to appropriately digested AcNPV DNA (as in Fig. 3); digestion with another restriction enzyme; and hybridization of ³²P-labeled recombinant plasmid DNA to Southern blots of AcNPV digested with several enzymes (as in Fig. 4). The electrophoretic

FIG. 3. Examples of recombinant plasmids containing AcNPV DNA *Eco*RI fragments. Plasmid DNA prepared by acid-phenol extraction was digested with *Eco*RI and subjected to electrophoresis on a 0.8% agarose gel. Lane 6 contains fragments A to K of *Eco*RI digested AcNPV DNA. All other lanes contain digests of plasmids containing AcNPV DNA fragments as follows: (1) pAcEcoA14, *Eco*RI-A; (2) pAcEcoB20, *Eco*RI-B; (3) pAcEcoCX18, *Eco*RI-C; (4) pAcEcoD19, *Eco*RI-D; (5) pAcEcoE7, *Eco*RI-E; (7) pAcEcoF22, *Eco*RI-F; (8) pAcEcoGX2, *Eco*RI-G; (9) pAcEcoH5, *Eco*RI-H; (10) pAcEcoI16, *Eco*RI-I; (11) pAcEcoKRW12, *Eco*RI-K. The band common to all the digested plasmids is linear pBR322. Recombinant plasmid nomenclature is as described in Table 2.

mobility of a cloned fragment along with the colony hybridization data was usually enough to identify it as a specific viral fragment. However, restriction digestion and hybridization data were generally obtained for unambiguous identification and were critical in the construction of the physical map. Table 2 summarizes the results of hybridizations of ³²P-labeled recombinant plasmids to Southern blots of AcNPV DNA digests. Some of the clones, in addition to hybridizing to

FIG. 4. Hybridization of ³²P-labeled recombinant plasmids to Southern blots of AcNPV DNA digested with *Bam*HI (A), *Eco*RI (B), *Hind*III (C), and *Pst*I (D) restriction endonucleases. The ³²P probe is indicated at the top of each panel. Conditions of electrophoresis, blot transfer, and hybridization were as described in Materials and Methods. The results to this figure are summarized in Table 2.

TABLE 2. Summary of the results of hybridizations of ³²P-labeled recombinant plasmids to AcNPV DNA digests on nitrocellulose filters

³² P probe ^a	Hybridization ^b					
	<i>Bam</i> HI	<i>Eco</i> RI	<i>Hind</i> III	<i>Pst</i> I	<i>Xho</i> I	<i>Sma</i> I
pAcEcoA14	A, C	A	D, J, L, X	G, I, J, O	C/D, F, H, (J)	A
pAcEcoB20	B	B	F, G, P	B, D, N		A
pAcEcoCX18	A, B	C, X	A/B, Q	A, B	A, J	A, D, C
pAcEcoD19	A	D	C, H, S, W	A, C		
pAcEcoE7	A, E	E	A/B	C, L, M		
pAcEcoF22	A	F	A/B, I	E, H	A	A, D
pAcEcoFM3	A	F, M	A/B, E, I, N/O, U	E, H	A, E, L	
pAcEcoFX24	A, B	F, X	A/B, I, Q	A, B, E, H		
pAcEcoGX2	A, B	G, X	C, Q	A, B		
pAcEcoH5	B, D, E, G	H	A/B, K, (Q)	B, M	G, I, J	A, B
pAcEcoI16	B, C, F	I	F, N/O, T, V	D	C/D	A
pAcEcoL26	A	L	A/B, (L), (Q)	C, (J)		
pAcEcoN28	A	N	I, U	E	L, M	
pAcEcoP1	B	P	P, Q	B	J, K	A
pAcEcoQ27	A	Q	B, (L), (Q)	C, (J)		
pAcHindE3	A	J, K, M, T	E	E, F		
pAcHindG2	B	B	G	B, (O)		
pAcHindH10	A	D	H	C		B, C
pAcHindK1	B	H, S	K	B	I, J	
pAcHindM11	A	J	M	J, K		A
pAcHindQ7	B	P, S, X	Q, (L)	B, (J)		
pAcHindRU5	A	J, M, N	R, U	E, F, K		
pAcHindU13	A	M, N	U	E	L	
pAcBamE1	E	E, H	A/B	B, M		
pAcBamF2	F	I	F, T, V	D		A

^a Clones were named according to the fragments they were found to contain. For example, a clone containing the *Eco*RI-A fragment was named pAcEcoA14: p for plasmid, Ac for AcNPV DNA, Eco for the *Eco*RI restriction enzyme, A for the *Eco*RI-A fragment, and 14 for the isolate number.

^b Some clones in addition to hybridizing to the expected regions of the genome hybridized to other regions. Hybridization to additional regions is indicated by parentheses. A slant line indicates that it was not possible to distinguish between two fragments because of comigration in agarose gels.

the expected regions of the genome, hybridized to other regions. Most frequently, additional hybridization was to the *Hind*III-L and -Q regions with plasmids pAcEcoL26, pAcEcoQ27, and pAcHindQ7. Binding to these regions was also observed when hybridization conditions were more stringent (see Materials and Methods). This additional hybridization could have been due to reiteration of sequences between the *Hind*III-L and -Q regions of the genome.

Construction of a physical map of AcNPV-DNA. The circular genome is described in Fig. 5 as a map linearized at the *Eco*B and *Eco*I junction. This is in accordance with a convention proposed to baculovirus researchers investigating the genome of AcNPV (G. E. Smith and J. M. Vlak, personal communication). Comigrating restriction fragments are designated as a single letter in alphabetical order starting from the zero point (e.g., *Hind*-N and *Hind*-O). Positions on this map are defined as the percentage of the total length of the genome and were calculated from the size of each fragment (Table 1) together with the sets of data required to order the fragments.

The hybridizations detailed in Table 2 usually gave enough information to deduce the order and approximate position of each fragment. The physical map shown in Fig. 5 was derived from these hybridizations together with the following supportive data: (i) the calculated sizes of the restriction fragments (Table 1); (ii) reciprocal double digestions of viral DNA; (iii) digestions of cloned DNA fragments with other enzymes; (iv) digestions of isolated *Sma*I DNA fragments; and (v) partial digestion with *Hind*III of cloned and isolated DNA fragments. The following example illustrates how the order of the *Hind*III fragments F, V, T, and N was deduced. The recombinant plasmid pAcEcoI16 hybridized to (i) *Bam*HI-B, -C, and -F; (ii) *Eco*RI-I; (iii) *Hind*III-F, -N, -T, and -V; and (iv) *Pst*I-D (see Fig. 4). Digestion of pAcEcoI16 with *Hind*III resulted in intact *Hind*III-V and -T fragments. The clone pAcBamF2 hybridized to (i) *Bam*HI-F; (ii) *Eco*RI-I; (iii) *Hind*III-F, -T, and -V; and (iv) *Pst*I-D (Fig. 4). Digestion of pAcBamF2 with *Hind*III resulted in an intact *Hind*III-V fragment. These results, together with the calculated sizes of the restriction fragments were

FIG. 5. Physical map of AcNPV HR3 DNA for the enzymes *SmaI*, *BamHI*, *XhoI*, *PstI*, *HindIII*, *EcoRI*. The map was linearized at the junction of the *EcoRI*-I and -B fragments. Each cleavage site is indicated with a horizontal line and is labeled as a percentage of the genome. Comigrating restriction fragments are designated as a single letter in alphabetical order starting from 0.0% (e.g., *Hind*-N and -O).

enough to order the *HindIII* fragments as F, V, T, and N, and to map them with respect to the other fragments (Fig. 5).

Restriction fragments not previously reported, *HindIII*-W and -X and *EcoRI*-W and -X, were mapped as follows. *HindIII*-W was mapped between *HindIII*-C and -H because of the hybridization pattern for pAcEcoD19 (Table 2) and because an intact *HindIII*-W fragment was found when *SmaI*-C was digested with *HindIII*. *HindIII*-X was mapped between *HindIII*-D and -J because of the hybridization data for pAcEcoA14, together with the partial digestion data of pAcEcoA14 with *HindIII*, and because *HindIII*-X was generated when *PstI*-O was digested with *HindIII*. *EcoRI*-W was mapped between *EcoRI*-G and -D because digestion of *SmaI*-C and *XhoI*-B generated *EcoRI*-W. *EcoRI*-X was mapped between *EcoRI*-S and -P because of the hybridization characteristics of pAcHindQ7 and clones containing *EcoRI*-X and because digestion of pAcHindQ7 with *EcoRI* gave *EcoRI*-X.

The physical map derived here is quite similar to the E2 physical map of Smith and Summers (12) for the enzymes *SmaI*, *BamHI*, *XhoI*, and *EcoRI* except for the positions of *EcoRI*-S and -P and *XhoI*-J and -K. The positions for these fragments in Fig. 5 were derived from the hybridization characteristics for the clones pAcEcoH5, pAcHindK1, and pAcEcoP1 (Table 2).

DISCUSSION

Sequences representative of more than 95% of the AcNPV genome were cloned as *EcoRI*, *HindIII*, and *BamHI* restriction fragments in the plasmid vector pBR322. The cloned fragments were identified by their electrophoretic mobility with respect to appropriately restricted AcNPV DNA (Fig. 3) and by hybridization of nick-translated recombinant plasmid to Southern blots of AcNPV DNA restricted with *BamHI*, *EcoRI*, *HindIII*, *PstI*, *XhoI*, and *SmaI* endonucleases (Fig. 4). The hybridization data obtained from these analyses (Table 2), together with restriction analysis of the individual *SmaI* fragments, also served to confirm the physical maps of our wild-type strain of AcNPV (HR3) with respect to those previously published (8, 11) for the restriction enzymes *SmaI*, *BamHI*, *XhoI*, *EcoRI*. These data were also used to establish the complete physical maps for *PstI* and *HindIII* endonuclease-generated DNA fragments.

The physical map for *HindIII* endonuclease was of special interest because differences between our HR3 strain, the E2 variant of Smith and Summers (11), and L1 strain of Miller and Dawes (9) were evident after *HindIII* digestion (Fig. 2). The only difference between HR3 and E2 is that the *HindIII*-L' fragment of E2 is about 200 bp smaller than that of HR3. However, this difference is small enough not to be reflected in

the physical maps. The L1 strain has the same-sized L' fragment as the E2 variant and has an extra *Hind*III site in the *Hind*III-A fragment generating *Hind*III-B' and -H' fragments when compared with HR3 and E2. That is, the *Hind*III-B' and -H' fragments of the L1 strain appear to consist of the same region of the genome as the *Hind*III-A fragment of HR3 and E2. The extra *Hind*III site in the L1 genome is probably at the 78 to 79% region on the HR3 genome (Fig. 5).

The data presented demonstrate that fragments not previously reported are generated: an *Xho*I-N fragment of about 350 bp; an *Eco*RI-X fragment of about 500 bp; and *Hind*III-V, -W, and -X fragments of about 900, 800, 800 bp, respectively. Each of these fragments was found upon digestion of the E2 and L1 strains. Except for *Xho*I-N fragment, the locations of these W fragments and *Eco*RI-W are included in the physical map (Fig. 5).

The additional hybridizations (Table 2) suggest that *Hind*III-Q and -L regions contain common sequences. This possibility is supported by the following observations. Restriction fragments of AcNPV DNA appear to occur at one molar frequency on the basis of the ethidium bromide stain (Fig. 1). However, hybridization of nick-translated AcNPV DNA to Southern blots of AcNPV DNA digests confirmed the general pattern of equivalence except that *Hind*III-L and *Pst*I-J fragments hybridized as though they were double bands (Fig. 4). Both fragments correspond to the same location on the physical map. This binding pattern was not seen with other enzyme digests because of the size or disposition of the fragments in that region; i.e., *Sma*I-A, *Bam*HI-A, and *Eco*RI-A are too large and *Xho*I-F and -H fragments migrate too closely to the -G and -I fragments to detect any increase in hybridization. For the same reasons, it is difficult to see the effect of additional hybridizations to the *Hind*III-Q region of the genome. However, in well-resolved blots, *Hind*III-Q, *Eco*RI-P, and *Xho*I-J also hybridize as though each was a double band.

The availability of a library of AcNPV DNA cloned sequences will make the analysis of the viral genome easier as large amounts of these sequences can now be generated. Our current work indicates that the cloned fragments will be particularly useful as specific probes for hybridization selection of mRNA species in the development of a transcription map. The cloned fragments will also be useful in determining the map location of AcNPV mutants by marker rescue.

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