Spodoptera frugiperda Nuclear Polyhedrosis Virus Genome: Physical Maps for Restriction Endonucleases *Bam*HI and *Hind*III

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The physical map for the genome of *Spodoptera frugiperda* nuclear polyhedrosis virus was constructed for restriction endonucleases *Bam*HI and *Hind*III. The ordering of the restriction fragments was accomplished by cross-blot hybridization of *Bam*HI, *Hind*III, and *Eco*RI fragments. The alignment of the *Hind*III fragments within the *Bam*HI map was achieved by double digestion with the two restriction endonucleases followed by cross-blot hybridization. The results showed that the viral genome consisted of mainly unique sequences. In addition, the circular nature of the viral genome was reaffirmed.

Spodoptera frugiperda nuclear polyhedrosis virus (SF NPV) is a member of the baculovirus group with a double-stranded, circular, supercoiled DNA genome (16). The molecular weight of the DNA molecule has been determined to be about 80×10^6 by electron microscopy and restriction endonuclease analysis in this laboratory (manuscript submitted), in reasonable agreement with previous reports by other investigators (1, 2, 4, 7, 8, 16).

Because of its relatively large size and complexity, the construction of a restriction map of the viral genome should greatly facilitate the study of its molecular biology. It would allow investigators to study virus recombinants and map the crossover sites (14, 17). The comparison of different virus strains by restriction endonuclease analysis (9, 13) is more meaningful when it is possible to locate the regions showing the greatest genetic variation between virus strains. Even more important, it is essential for the study of gene transcription and regulation in both permissive and non-permissive cells. In this paper, a physical map of the SF NPV genome was constructed with the restriction endonucleases *Bam*HI and *Hind*III.

MATERIALS AND METHODS

Virus purification. The partial purification of polyhedra of the Ohio strain of SF NPV was performed at the Southern Grain Insects Laboratory from the lysate of virus-infected Fall army worm larvae in Tifton, Ga. Subsequently, this suspension of polyhedra was washed by repeated centrifugation in a Sorvall RC-5 refrigerated centrifuge at 5,000 rpm for 5 min and resuspension in distilled water until the supernatant fluid was clear. After the last centrifugation, the

polyhedra were suspended in a freshly prepared solution of 0.1 M Na₂CO₃ for 15 to 20 min at room temperature and centrifuged for 5 min at 5,000 rpm. This procedure was repeated two to three times until the alkaline degradation of the polyhedra was complete. The supernatant from each centrifugation was pooled, layered gently onto 20 to 60% (wt/wt) sucrose gradients made in TBS (0.15 M NaCl-0.05 M Tris-hydrochloride, pH 7.4) and centrifuged in an SW-27 rotor for 75 min at 25,000 rpm and 4°C. The multiple virus bands in each gradient were collected through the bottom of each tube, dialyzed against TBS overnight, and stored at 4°C. If a more concentrated virus preparation is desired, the virions may be pelleted by centrifugation and resuspended in an appropriate buffer.

Extraction of viral DNA. A concentrated suspension of gradient-purified virions was digested at 55°C for 1.5 h in a proteinase K digestion mixture (1% sodium dodecyl sulfate-5 mM CaCl₂-1 mM EDTA-100 µg of proteinase K per ml). When pronase (1 mg/ml) was substituted for proteinase K, the digestion was carried out at 37°C.

The digested mixture was carefully layered onto preformed 10 to 30% (wt/vol) sucrose gradients in TBS containing 1 mM EDTA and centrifuged in SW-27 rotors at 18,000 rpm for 18 h in a Beckman L3-40 ultracentrifuge. Fractions (1 ml) were collected through the top by pumping a 60% sucrose solution through the bottom of the tube. The DNA-containing fractions as determined by UV absorption were pooled, dialyzed against 0.05 M Tris-hydrochloride (pH 7.4)-1 mM EDTA buffer overnight and stored at 4°C.

Alternatively, a modification of the method of Radloff et al. (10) was used. The proteinase K-digested mixture was diluted with 0.01 M Tris-hydrochloride (pH 8.0)-0.01 M EDTA buffer containing ethidium bromide at a concentration of 200 µg/ml. Cesium chloride was added to the solution until the density was 1.59 g/ml. Centrifugation was carried out in a VI-

50 rotor at 36,000 rpm for 24 h in a Beckman ultracentrifuge. The DNA-containing fractions were visualized by their fluorescence in UV light and collected. The ethidium bromide was removed by extraction with isoamyl alcohol, and the DNA fractions were dialyzed against 0.05 M Tris-hydrochloride (pH 7.4)–0.001 M EDTA buffer overnight and stored at 4°C or further purified by sedimentation through a sucrose gradient as previously described.

If intact DNA molecules were not required, the DNA solution could be extracted with phenol, and the DNA could be precipitated with cold ethanol at –20°C and redissolved in an appropriate buffer.

Restriction enzyme digestion of viral DNA. Purified viral DNA was digested with *EcoRI*, *BamHI*, or *HindIII* in a solution containing 0.05 M Tris-hydrochloride (pH 7.4)–0.01 M $MgCl_2$ –0.10 M NaCl–0.006 M β -mercaptoethanol. Incubations were generally for 3 h at 37°C, and sufficient enzyme was added for complete digestion within this period. When partial digestion of the viral DNA became necessary for mapping purposes, the same amount of restriction enzyme was added, but incubation was carried out at 10°C for between 10 and 30 min. The reaction was stopped by adding EDTA to a final concentration of 10 mM. The restriction enzyme was then heat inactivated at 70°C for 20 min.

The restriction enzyme *EcoRI* was purified from *Escherichia coli* strain Ry13 (3), *HindIII* was purchased from Bethesda Research Laboratories, Inc., Rockville, Md., and *BamHI* was purified from *Bacillus amyloliquefaciens* H (19; L. A. Smith and J. G. Chirikjian, Fed. Proc. 36: 908, 1977).

In vitro labeling of DNA. For critical determinations of the molecular weights and stoichiometry of DNA fragments by electrophoresis in agarose gels, the DNA restriction fragments were end labeled in vitro with avian myeloblastosis virus reverse transcriptase obtained from G. E. Houts of the Life Sciences Institute, St. Petersburg, Fla. Approximately 10 μ Ci of [α -³²P]dATP (450 Ci/mmol; ICN Pharmaceuticals) were lyophilized in a small tube and redissolved in a reaction mixture containing 20 to 25 μ g of DNA restriction fragments per ml, 100 mM NaCl, 50 mM Tris-hydrochloride (pH 7.4), 6 mM β -mercaptoethanol, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1 mM dCTP, 0.1 mM dGTP, and 0.1 mM dTTP. Reverse transcriptase (15 U) was added, and incubation was carried out at 4°C for 20 to 30 min. The reaction was stopped by heat inactivating the enzyme in a 70°C water bath for 20 min. The mixture was then treated with proteinase K (50 μ g/ml) for 30 min at 37°C before gel electrophoresis.

For cross-blot hybridization experiments, labeling was done with DNA polymerase I instead of reverse transcriptase. The procedure, a modification of the method of Rigby et al. (12), was essentially the same as that described in the preceding paragraph, except that 5 μ l of DNA polymerase I was used, the $MnCl_2$ was not needed, and incubation was carried out at 14°C for 30 min. It was found that this procedure can be adopted for end labeling DNA restriction fragments as well. The DNA polymerase I used in these experiments was purified by the method of Richardson et al. (11) as modified by Jovin et al. (6).

Agarose gel electrophoresis of DNA frag-

ments. The DNA restriction fragments were fractionated by electrophoresis through 0.7% agarose (SeaKem) gels. Agarose was dissolved in E buffer (0.04 M Tris-hydrochloride–0.02 M sodium acetate–1 mM EDTA, pH 7.2) and allowed to solidify on a glass plate to form a horizontal slab gel (6 by 200 by 250 mm). For cross-blot hybridization experiments, slightly wider gels (6 by 250 by 250 mm) were used. Sample slots of suitable sizes were formed by inserting a plexiglas comb at one end of the gel before gel formation was complete. DNA samples were loaded into the slots with tracer amounts of a bromophenol blue solution made up in E buffer with 30% glycerol.

The DNA was electrophoresed at a constant voltage of 100 V at 25°C for about 18 h until the bromophenol blue marker reached the end of the gel.

Visualization of DNA fragments. When unlabeled DNA samples were electrophoresed, the gel was stained with ethidium bromide (20 μ g/ml in E buffer) for 1 h, and the DNA fragments could be visualized under UV light and photographed with a Polaroid MP-4 camera with an orange filter under the lens.

In the case of labeled DNA samples, the gel was dehydrated by vacuum on a gel dryer and exposed to Kodak X-Omat R X-ray film for 24 to 48 h. The positions of the DNA fragments were thus recorded on the developed film. For molecular weight measurements, lambda DNA digested with *EcoRI*, *BamHI*, or *HindIII* was used for molecular weight markers. The migration distances of the DNA fragments as recorded on film were determined by a Hewlett Packard 9825A calculator and digitizer programmed to calculate the DNA fragment sizes in kilobases when suitable molecular weight markers were provided. Densitometer tracings of the autoradiographs were done with a Joyce Loeb microdensitometer. The molar ratios of the DNA fragments, being proportional to the areas under the peaks, were then determined by cutting out and weighing the corresponding peaks on these tracings.

Cross-blot hybridization. To detect homology between different fragments, the Hutchison cross-blot hybridization technique (C. Hutchison, personal communication) was used. A "cold" restriction digest was run across the entire width of a square slab gel (6 by 250 by 250 mm) using 10 to 15 μ g of viral DNA per gel. The positions of the DNA bands could be photographed under UV light after staining with ethidium bromide. The DNA in the gel was then depurinated partially with 0.25 M HCl, cleaved and denatured with 0.5 M NaOH–1 M NaCl, and neutralized in 1 M Tris-hydrochloride (pH 7.4)–1.5 M NaCl to improve the efficiency of transfer of large DNA fragments onto a nitrocellulose sheet (18). The actual transfer was done in 6 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature for 8 h by the procedure of Southern (15) with slight modifications. The nitrocellulose sheet (Bethesda Research Laboratories) was then air dried briefly and baked in vacuum at 80°C for 3 h.

A ³²P-labeled restriction digest was run and prepared for transfer as described above. The nitrocellulose sheet with the immobilized DNA was placed on top of the radioactive gel such that each cold band bound to the nitrocellulose sheet intersected each

"hot" band in the gel at right angles. The hot band patterns were then transferred and directly hybridized at right angles to the unlabeled DNA bands on the nitrocellulose sheet in hybridization buffer (6× SSC-0.1% sodium dodecyl sulfate) at 65°C for 8 h in a humidified oven. All components of the system except the gel were preheated to 65°C before the transfer and hybridization step. Finally, the nitrocellulose sheet was washed as described by Jeffreys and Flavell (5), blotted dry, wrapped in a protective plastic freezer bag, and exposed to Kodak X-Omat R X-ray film for 3 days. A Du Pont Lightning-Plus intensifier screen was often used to shorten the exposure time. After development, spots of developed silver grains should mark the intersection points of DNA fragments sharing common sequences.

RESULTS

Restriction enzyme cleavage patterns. The SF NPV genome was cleaved into 8, 15, and 25 fragments by the restriction enzymes *Bam*HI, *Hind*III, and *Eco*RI, respectively (Fig. 1). The estimated molecular weights of the restriction fragments (see Table 6) have already been reported (manuscript submitted). As described in a later section, slight corrections to the molecular weights of *Bam*HI fragments were made. It is seen that there are four sets of comigrating fragments in the *Eco*RI digest, one in the *Hind*III digest, and none in the *Bam*HI digest. Thus, it was decided that the construction of restriction maps for *Hind*III and *Bam*HI should be attempted initially because they would allow less ambiguous assignment of transcription patterns and regions of genetic variation between virus strains by using the Southern blot hybridization technique.

Construction of a *Bam*HI restriction map by cross-blot hybridization. The first step in the mapping procedure was to determine whether there were repeat sequences within the viral genome which could complicate the interpretation of hybridization data. This was accomplished by the cross-blot hybridization of cold *Bam*HI fragments to ³²P-labeled *Bam*HI fragments (Fig. 2). Spots were found along the diagonal only. Thus, there were probably no long repeat sequences present in the viral genome.

Partial digestion of SF NPV DNA should produce DNA fragments consisting of two or more "complete" restriction fragments linked together. By hybridizing ³²P-labeled *Bam*HI fragments to unlabeled, partially digested *Bam*HI fragments immobilized on a nitrocellulose sheet, the linkages between different *Bam*HI restriction fragments could be deduced (Fig. 3 and Table 1). The deduction of the single linkages from fragments 3, 4, 5, and 6 are quite obvious. Fragment 2 has four spots, indicating sequence homologies with *Bam*HI fragments B,

FIG. 1. Cleavage patterns of SF NPV DNA by the restriction endonucleases *Bam*HI, *Hind*III, and *Eco*RI. The restriction fragments were labeled with [α -³²P]dATP in the presence of DNA polymerase I. The partial fragment between fragments D and E in the *Eco*RI digest was the result of slightly incomplete digestion.

E, F, and G. One of the two comigrating fragments must be fragment B, because one spot is on the diagonal where the labeled and unlabeled B fragments should intersect. Thus, the three *Bam*HI fragments E, F, and G must be linked together to form the other comigrating fragment. Since fragment E is directly linked to both fragments F and G, the three fragments must be linked in the manner indicated in Table 1, i.e., F-E-G. To sum up, the data in Table 1 demonstrate linkages between five *Bam*HI fragments, and the deduced ordering is H-C-F-E-G.

To complete the ordering of *Bam*HI restriction fragments, ³²P-labeled *Hind*III fragments were hybridized to unlabeled *Bam*HI fragments in another cross-blot hybridization experiment

FIG. 2. Autoradiograph of the patterns resulting from cross-blot hybridization between ^{32}P -labeled and cold BamHI fragments of SF NPV DNA. A ^{32}P -labeled BamHI digest was run side by side with the cold BamHI digest and transferred to the nitrocellulose sheet. The identification of the cold BamHI fragments was thus facilitated by noting the position of the corresponding transferred ^{32}P -labeled fragment.

FIG. 3. Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P -labeled BamHI fragments and a cold partial BamHI digest of SF NPV DNA. The ^{32}P -labeled fragments of the partial digest was run side by side with the cold fragments and transferred to the nitrocellulose sheet to facilitate the identification of the unlabeled fragments.

TABLE 1. Cross-blot hybridization of ^{32}P -labeled *Bam*HI fragments to unlabeled *Bam*HI partial digestion products

Partial digestion products	<i>Bam</i> HI fragments with homologous sequences	Linkage groups
1	A	
2 ^a	B	
	E, F, G	F-E-G
3	C, F	C-F
4	E, F	F-E
5	C, H	H-C
6	E, G	E-G
7	C	
8	D	
9	E	
10	F	
11	G	
12	H ^b	
13	H	

^a Two comigrating fragments.

^b Fragment 12 probably corresponds to *Bam*HI fragment H linked to a short fragment since fragment 13 is on the diagonal marking the intersection points of identical cold and ^{32}P -labeled *Bam*HI fragments. The short fragment was not observed in the 0.7% agarose gels because its small size allowed it to migrate ahead of the bromophenol blue marker.

(Fig. 4 and Table 2). If a *Hind*III fragment shares sequence homology with only one *Bam*HI fragment as is the case with fragments E, G, I₁, J, K, L, M, and N, the *Hind*III fragment in question must be entirely within the *Bam*HI fragment involved. However, when a *Hind*III fragment shares sequence homology with two or more *Bam*HI fragments, these *Bam*HI fragments must be linked together with the *Bam*HI restriction sites at the linkage points lying within the *Hind*III fragment involved. This is illustrated in Table 2 by the *Hind*III fragments A, B, C, D, F, H, and I₂. Thus, with *Bam*HI fragment B linked to fragments H and D and *Bam*HI fragment A linked to fragments D and G, the ordering of the *Bam*HI restriction fragments could be completed (Fig. 5a).

Construction of a *Hind*III restriction map by cross-blot hybridization. Using the data presented in Table 2 in combination with the known ordering of the *Bam*HI restriction fragments, a partial ordering of the *Hind*III fragments could be deduced (Fig. 5b). Where uncertainties in ordering existed, the fragments involved were included in one block. This is true for *Hind*III fragments E, G, and L, which lie totally within *Bam*HI fragment A, *Hind*III fragments I₁ and M, which lie within *Bam*HI fragment D, and *Hind*III fragments K and N, which lie within *Bam*HI fragment C.

To clear up these ambiguities, ^{32}P -labeled

FIG. 4. Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P -labeled *Hind*III fragments and cold *Bam*HI fragments of SF NPV DNA. The smearing of the spots was due to the slight overloading of the sample slots during gel electrophoresis of the cold *Bam*HI fragments.

TABLE 2. Cross-blot hybridization of ^{32}P -labeled *Hind*III fragments to unlabeled *Bam*HI fragments

<i>Hind</i> III fragment	<i>Bam</i> HI fragments with homologous sequences	Linkage groups for <i>Bam</i> HI
A	B, D	D-B
B	C, E, F	C-F-E
C	E, G	E-G
D	A, G	G-A
E	A	
F	A, D	A-D
G	A	
H	B, H	B-H
I ₁	A ^a , C, D, H	D
I ₂		H-C
J	B	
K	C	
L	A	
M	A ^a , D	
N	C	

^a These two spots suggest a possible sequence homology between parts of *Bam*HI fragment D and *Bam*HI fragment A. The relevant sequences within *Bam*HI fragment D are located near the junction between *Hind*III fragments M and I₁. The sequence within *Bam*HI fragment A is located within *Hind*III fragment L (Fig. 8 and Table 5).

*Eco*RI fragments were hybridized to unlabeled *Hind*III fragments in a cross-blot hybridization experiment (Fig. 6 and Table 3). The linkage

groups were deduced by the reasoning described above. In the assignment of linkages deduced from comigrating *EcoRI* fragments C₁ and C₂, it is seen that *HindIII* fragments D and E must be linked because fragments H and I₂ are known to be linked and located well away from both D and E. However, the assignments involving *EcoRI* comigrating fragments D₁, D₂, and D₃ are more speculative. Fortunately, the ordering of the *HindIII* fragments involved is already pre-

FIG. 5. a, Physical map of SF NPV genome for *Bam* HI; b, partial physical map of SF NPV genome for *Hind* III; c, physical map of SF NPV genome for *Hind* III.

cisely known. With the additional information in Table 3, the *Hind* III restriction map can now be deduced. It is seen that *Hind* III fragment E is linked to both fragments D and L. Thus, it is easy to reason that the fragments D, E, F, G, and L are joined in the order D-E-L-G-F. Similarly, knowing that fragment I₁ is linked to both fragments A and M, one can deduce that the fragments A, F, I₁, and M are linked in the order F-M-I₁-A. Finally, with *Hind* III fragment K linked to fragment B, the fragments B, I₂, K and N can be unambiguously joined as I₂-N-K-B. The resulting restriction map for *Hind* III is shown in Fig. 5c.

Double digestion of SF NPV DNA by *Bam* HI and *Hind* III. The double digestion of SF NPV DNA by *Bam* HI and *Hind* III serves a threefold purpose. First, it provides an independent confirmation of the restriction map deduced from cross-blot hybridization. Second, it allows the precise alignment of *Hind* III restriction fragments within the *Bam* HI map or vice versa. Last, it gives better estimates of the molecular weights of large DNA fragments because these fragments are cleaved by the second en-

FIG. 6. Autoradiograph of the patterns resulting from the cross-blot hybridization between ³²P-labeled *EcoRI* fragments and cold *Hind* III fragments of SF NPV DNA.

TABLE 3. Cross-blot hybridization of ^{32}P -labeled *EcoRI* fragments to unlabeled *HindIII* fragments

<i>EcoRI</i> fragment	<i>HindIII</i> fragments with homologous sequences	Linkage groups for <i>HindIII</i>
A	F, G, L	L-G-F
B	A, I	I ₁ -A
C ₁ }	D, E, H, I	D-E, H-I ₂
C ₂ }		
D ₁ }		
D ₂ }		
D ₃ }	B, C, D, E	B-C, C-D, E
E		
F		
G		
H	A	I ₂ -N-K
I	C	
J ₁ }	I, K, N	
J ₂ }	F	
K	B	E-L
L	C, J	
M	M	
N ₁ }	E, L	
N ₂ }	A	J-H
O	F, K	
P	H, J	
Q	F	
R	A, J	A-J
S	B, K	
T	B	K-B
	M, I	
		M-I ₁

zyme into smaller fragments that are within the range of molecular weights provided by the markers. In our case, this is applicable to the *Bam*HI fragments A and B.

SF NPV DNA was first digested with *Hind*III and then with *Bam*HI, end labeled with [α - ^{32}P]dATP, and electrophoresed in a 0.7% agarose gel. A complete *Hind*III digest was run at the same time so that the fragments that were cleaved by *Bam*HI could be identified. The molecular weights and stoichiometry of the restriction fragments were estimated as described above. The *Bam*HI and *Hind*III double-digest patterns are shown in Fig. 7. It is seen that the *Hind*III fragments B, C, D, F, H, and I₂ have disappeared. In addition, 13 new fragments are present. Referring to the restriction maps in Fig. 5, this is exactly what is expected, with *Hind*III fragments C, D, F, H, and I₂ each being cleaved once and fragment B cleaved twice. Fragment A should also be cleaved once. This is confirmed by its slight shift in position to one corresponding to a lower molecular weight. The cleaved-off piece of DNA is probably so short that it is running ahead of the bromophenol blue marker and has migrated out of the gel. The estimated molecular weights of these double-digestion products are presented in Table 4.

Alignment of *Hind*III fragments within the *Bam*HI map. To achieve the alignment of

*Hind*III fragments within the *Bam*HI map, ^{32}P -labeled *Hind*III fragments were hybridized to unlabeled DNA fragments from a *Hind*III-*Bam*HI double digestion in a cross-blot hybridization experiment (Fig. 8). The ^{32}P -labeled *Hind*III fragments will hybridize to the double-digest fragments that share their sequence homology, thus allowing the identification of specific double-digest fragments that form the original *Hind*III restriction fragments (Table 5). The location of *Bam*HI sites within the *Hind*III fragments can then be calculated from the estimated molecular weights of the double-digestion fragments listed in Table 4. The alignment of *Hind*III fragments within the *Bam*HI map was thus accomplished. For example, the *Hind*III fragment D, made up of fragments 4 and 18 of the double digest, spans the *Bam*HI fragments

FIG. 7. Cleavage patterns of SF NPV DNA by *Hind*III and (*Hind*III + *Bam*HI). The restriction fragments were labeled with [α - ^{32}P]dATP in the presence of DNA polymerase I.

TABLE 4. *Molecular weights of HindIII-BamHI double-digest fragments*

Fragment	Mol wt (10 ⁶)
1	11.1
2	7.8
3	7.0
4	6.7
5	6.4
6	5.3
7	3.9
8	3.8
9	3.7
10 (2 comigrating fragments)	3.5
11	3.1
12	2.7
13	2.4
14	1.9
15	1.8
16	1.8
17	1.6
18	1.5
19	1.4
20	0.72
21	0.59

A and G. Since fragment 4 is too long to fit into *Bam*HI fragment G, it must be within *Bam*HI fragment A, whereas fragment 18 lies within *Bam*HI fragment G. Similar deductions can be made, and the results are summarized in the third column of Table 5.

During the initial alignment, it was found that the previously determined molecular weights of *Bam*HI fragments A and B were too low. Thus, using the estimated molecular weights of the double-digestion fragments (Table 4), more accurate values of 24.1×10^6 and 18.5×10^6 , respectively, were assigned to the *Bam*HI fragments A and B and used in the construction of the final restriction map of *Bam*HI. The molecular weights of the other *Bam*HI fragments were proportionally adjusted so that the size of the whole DNA molecule was again 82.5×10^6 . The molecular weights of the *Hind*III and *Bam*HI restriction fragments used in the map are presented in Table 6. The restriction map of SF NPV DNA for *Bam*HI and *Hind*III is shown in Fig. 9.

FIG. 8. Autoradiograph of the patterns resulting from the cross-blot hybridization between ³²P-labeled *Hind*III fragments and cold *Hind*III and *Bam*HI double-digested fragments of SF NPV DNA.

FIG. 9. Physical maps of the SF NPV genome for the restriction endonucleases *Bam*HI and *Hind*III. The *Bam*HI fragment A is arbitrarily placed at the origin of the linear map for the sake of simplicity. The actual map should be circular.

DISCUSSION

The restriction map of the SF NPV DNA genome is presented in Fig. 9 in linear rather than circular form for the sake of simplicity. However, the restriction enzyme mapping data confirm the circular nature of the DNA molecule. The SF NPV genome consists of mainly unique sequences. This is in agreement with the results from Kelly's reassociation kinetics experiments (7) and our previous partial denaturation mapping data (manuscript submitted). Our cross-blot hybridization data (Fig. 4 and 8, Tables 2 and 5) indicate that the only possible repeat sequences of any appreciable length may be those located in *Hind*III fragment L and those near the junction of *Hind*III fragments M and I₁. However, one cannot exclude the possibility of additional short repeat sequences within

TABLE 5. Sequence homology between the *Hind*III-*Bam*HI double-digestion products and *Hind*III or *Bam*HI restriction fragments

Double-digest fragment	<i>Hind</i> III fragments with homologous sequences ^a	<i>Bam</i> HI fragments with homologous sequences ^b
1	A	B
2	C	E
3	E	A
4	D	A
5	B	F
6	G	A
7	H	B
8	I ₁	D
9	J	B
10	F	D
(2 comigrating fragments)	K	C
11	B	C
12	F	A
13	L	A
14	I ₂	H
15	I ₂	C
16	L ^c , M	A ^c , D
17	C	G
18	D	G
19	N	C
20	B ^d	E
21	H ^d	H

^a From cross-blot hybridization data.

^b From restriction map alignment.

^c The spot at the point of intersection of fragment 16 and fragment L suggests a possible sequence homology between *Hind*III fragments L and M (i.e., fragment 16 in the double digest). This is already indicated in Table 2.

^d These are deduced by comparing the molecular weights of complete *Hind*III fragments and their double-digestion products as well as restriction map positions.

TABLE 6. Molecular weights of the *Bam*HI, *Hind*III, and *Eco*RI restriction fragments

Fragment	Mol wt (10 ⁶)
<i>Bam</i> HI	
A	24.1
B	18.5
C	10.2
D	9.1
E	8.8
F	6.0
G	3.2
H	2.6
<i>Hind</i> III	
A	11.3
B	10.2
C	9.6
D	8.4
E	6.9
F	6.1
G	5.3
H	4.4
I ₁	3.9
I ₂	3.9
J	3.6
K	3.5
L	2.4
M	1.8
N	1.4
<i>Eco</i> RI	
A	8.1
B	7.1
C ₁	6.7
C ₂	6.7
D ₁	6.1
D ₂	6.1
D ₃	6.1
E	4.8
F	3.8
G	3.3
H	2.4
I	2.4
J ₁	2.0
J ₂	2.0
K	1.5
L	1.4
M	1.3
N ₁	1.2
N ₂	1.2
O	1.2
P	1.1
Q	1.0
R	0.90
S	0.87
T	0.82

parts of the genome where no *Bam*HI, *Hind*III, or *Eco*RI restriction sites are present.

The restriction maps for *Bam*HI and *Hind*III sites should provide sufficient sectioning of the SF NPV genome to facilitate initial studies of

transcription patterns and genetic variations between virus strains. The relatively precise locations of the *Bam*HI and *Hind*III restriction sites are confirmed by our double-digestion data where good placement of the sites for the second enzyme within the sites of the first enzyme is possible. If a specific region of the genome needs to be studied more closely, restriction mapping with a third or fourth enzyme such as *Eco*RI or *Xba*I may be attempted to provide an even smaller sectioning of the viral genome.

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LITERATURE CITED

1. Bud, H. M., and D. C. Kelly. 1977. The DNA contained by nuclear polyhedrosis viruses isolated from four *Spodoptera* Spp. (*Lepidoptera*, *Noctuidae*): genome size and configuration assessed by electron microscopy. *J. Gen. Virol.* **37**:135-143.
2. Burgess, S. 1977. Molecular weights of *Lepidopteran* baculovirus DNAs: derivation by electron microscopy. *J. Gen. Virol.* **37**:501-510.
3. Greene, P. J., M. C. Bethlach, H. M. Goodman, and H. W. Boyer. 1974. The *Eco*RI restriction endonuclease, p. 87-105. In R. B. Wickner (ed.), *Methods in molecular biology*, vol. 7. Marcel Dekker, New York.
4. Harrop, K. A., C. C. Payne, and J. S. Robertson. 1977. The properties of three Baculoviruses from closely related hosts. *Virology* **79**:14-31.
5. Jeffreys, A. J., and R. A. Flavell. 1977. A physical map of the DNA regions flanking the rabbit β -globin gene. *Cell* **12**:429-439.
6. Jovin, J. M., P. T. Englund, and L. L. Bertsch. 1969. Enzymatic synthesis of DNA. XXVI. Physical and chemical studies of a homogenous DNA polymerase. *J. Biol. Chem.* **244**:2996-3008.
7. Kelly, D. C. 1977. The DNA contained by nuclear polyhedrosis viruses isolated from four *Spodoptera* Sp. (*Lepidoptera*, *Noctuidae*): genome size and homology assessed by DNA reassociation kinetics. *Virology* **76**:468-471.
8. Knudson, D. L., and T. W. Tinsley. 1978. Replication of a nuclear polyhedrosis virus in a continuous cell line of *Spodoptera frugiperda*: partial characterization of the viral DNA, comparative DNA-DNA hybridization, and patterns of DNA synthesis. *Virology* **87**:42-57.
9. Miller, L. K., and K. P. Dawes. 1978. Restriction endonuclease analysis for the identification of baculovirus pesticides. *J. Virol.* **35**:411-421.
10. Radloff, R., W. Bauer and J. Vinograd. 1967. A dye-buoyant density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* **57**:1514-1521.
11. Richardson, C. C., C. L. Schildkraut, H. V. Aposhian and A. Kornberg. 1964. Enzymatic synthesis of deoxyribonucleic acid. XIV. Further purification and properties of DNA polymerase of *E. coli*. *J. Biol. Chem.* **239**:222-232.
12. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**:237-251.
13. Smith, G. E., and M. D. Summers. 1979. Restriction maps of five *Autographa californica* MNPV variants, *Trichoplusia ni* MNPV, and *Galleria mellonella* MNPV DNAs with endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, and *Eco*RI. *J. Virol.* **30**:828-838.
14. Smith, G. E., and M. D. Summers. 1980. Restriction map of *Rachiplusia on* and *Rachiplusia ou*-*Autographa californica* baculovirus recombinants. *J. Virol.* **33**:311-319.
15. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503-517.
16. Summers, M. D., and D. L. Anderson. 1973. Characterization of nuclear polyhedrosis viral DNAs. *J. Virol.* **12**:1336-1346.
17. Summers, M. D., G. E. Smith, J. D. Knell, and J. P. Burand. 1980. Physical maps of *Autographa californica* and *Rachiplusia ou* nuclear polyhedrosis virus recombinants. *J. Virol.* **34**:693-703.
18. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. U.S.A.* **76**:3683-3687.
19. Wilson, G. A., and F. F. Young. 1975. Isolation of a sequence-specific endonuclease from *Bacillus amyloliquefaciens* H. *J. Mol. Biol.* **97**:123-125.