Molecular cloning and physical mapping of the genome of Bombyx mori nuclear polyhedrosis virus

Susumu Maeda* and Kei Majima†

Department of Entomology, University of California, Davis, California 95616, U.S.A.

A restriction fragment library which covered the entire genome of Bombyx mori nuclear polyhedrosis virus (BmNPV) was constructed using plasmid vectors. By analysis of cloned and viral DNA by double digestion with endonucleases and by hybridization techniques, a complete physical map of BmNPV was constructed for BamHI, EcoRI, HindIII, KpnI, PstI and SmaI. Five regions of repeated sequences containing EcoRI sites were also found and mapped.

Bombyx mori nuclear polyhedrosis virus (BmNPV) is a member of subgroup A of the Baculoviridae, which is characterized by rod-shaped enveloped virions containing a genome of circular double-stranded DNA. NPVs are generally characterized as either SNPVs or MNPVs depending on the number (single or multiple) of bundled nucleocapsids within a viral envelope embedded in polyhedra; however, this characteristic of BmNPV seems not to be confined to one form. Although BmNPV is listed as representative of SNPVs (Matthews, 1982), BmNPV also produces a MNPV form. In an established cell line, only SNPVs seem to be observed (Inoue & Mitsuhashi, 1984). In larvae most progeny viral particles (more than 90%) are of the S form and the ratio of S to M forms depends on the infected organ (Watanabe, 1975). After plaque purification, BmNPV still shows this heterogeneity and this ratio does not change significantly after passage in vivo and in vitro (S. Maeda, unpublished data).

Baculoviruses have been considered as viral insecticides for insect control (Granados & Federici, 1986), and BmNPV has been a major disease of the silkworm, B. mori, in sericulture (Horie & Watanabe, 1980). BmNPV and Autographa californica NPV (AcNPV) have been used for the expression of foreign genes in insect cells and in whole insects (Miller, 1988; Maeda, 1989a), but the BmNPV has the advantage of having a well studied and easy to use host, the silkworm (Maeda et al., 1985). Although many aetiological and histopathological studies have been conducted, little has been reported on the genetics of BmNPV at the molecular level.

Construction of a physical map of the viral genome using restriction endonucleases is essential for further experiments at the molecular level. Such maps of several other NPVs have been constructed for Autographa californica NPV and its variants (Miller & Dawes, 1979; Smith & Summers, 1979; Vlak, 1980; Cochrane et al., 1982; Brown et al., 1984), Anticarsia gemmatalis NPV (Johnson & Maruniak, 1989), Panolis flammea NPV (Possee & Kelly, 1988), Mamestra brassicae NPV (Wiegars & Vlak, 1984; Possee & Kelly, 1988), Orgyia pseudotsugata NPV (Chen et al., 1988), Heliothis zea SNPV (Knell & Summers, 1984), Spodoptera littoralis NPV (Crozier et al., 1989) and Spodoptera frugiperda NPV (Loh et al., 1981; Maruniak et al., 1984). In this report we describe construction in plasmids of a restriction fragment library of the entire BmNPV genome and construction of a physical map for several endonucleases. In addition, five areas of EcoRI site-rich repeated sequences were found and localized on the physical map.

A plaque-purified isolate, T3, of BmNPV (Maeda, 1984) was used for construction of the gene library. Polyhedral inclusion bodies propagated in the silkworm were dissolved in alkaline solution and the released virus particles were purified by ultracentrifugation (Kawarabata & Matsumoto, 1973). Viral DNA was extracted from these particles after treatment with proteinase K (Merck) in the presence of 1% SDS (Maeda, 1989b). The DNA was digested with EcoRI, HindIII, PstI, BamHI, KpnI or SmaI (New England Biolabs) under conditions recommended by the supplier. The cleaved fragments were separated on 0.7% agarose gels along with lambda phage DNA size markers using a Tris-acetate buffer system (Maniatis et al., 1982). The patterns, shown in Fig. 1, had none of the submolar bands which are

† Present address: Faculty of Agriculture, Tottori University, Tottori 680, Japan.

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Figure 1. Cleavage patterns of BmNPV DNA using the restriction endonucleases EcoRI, HindIII, PstI, BamHI, KpnI or Smal. Viral DNA was digested with these endonucleases and the cleaved fragments were separated on a 0.7% agarose gel. Lane marked λ/HindIII shows molecular size marker patterns in kbp. Each visible fragment was assigned a letter as shown.

sometimes found in the preparation of other NPVs (Miller & Miller, 1982). Molecular sizes of the fragments were estimated as shown in Table 1 by comparison with the migration of the size markers. The sizes of some of the larger fragments were estimated from the size of subcloned fragments described later. The size of the entire genome was estimated to be about 130 kbp by summing the sizes of the fragments generated by the restriction endonucleases. This estimated size is quite similar to that of AcNPV (Lee & Miller, 1978).

DNA fragments digested with EcoRI, HindIII, KpnI, PstI or BamHI were cloned into pBR322, pUC9 or pUC19. Two large EcoRI fragments, A and B, were first cloned into Charon 4A (Maniatis et al., 1982), digested with restriction endonucleases HindIII and/or BamHI and then subcloned into pUC plasmids. Most of the cloned DNA was authenticated by comigration in agarose gels with digested viral DNA. All EcoRI fragments larger than 500 bp were successfully cloned into plasmids. These EcoRI clones and additional clones of the 21 DNA fragments listed in Table 1 overlapped to cover the entire viral genome without breaks at any restriction sites.

To construct a physical map of the BmNPV genome, the cloned plasmid DNAs were used as probes and hybridized to viral DNA fragments. Viral DNA was digested with the same restriction endonucleases used for cloning and electrophoresed on agarose gels. The

| Table 1. Sizes (kbp) of BmNPV restriction fragments |
|-----------------------------------------------------|
| Fragment | EcoRI | HindIII | PstI | BamHI | KpnI | Smal |
|----------|-------|---------|------|-------|------|------|
| A        | 20-4* | 30-0    | 17-5*| 54    | 52   | 97   |
| B        | 20-1* | 17-0    | 17-5*| 36    | 48   | 25   |
| C        | 14-5* | 15-5*   | 17-0 | 22    | 28   | 8-0  |
| D        | 13-9* | 10-0    | 12-5*| 7-3*  | 1-8* |
| E        | 10-5* | 8-9     | 10-8*| 6-0*  |      |      |
| F        | 8-7*  | 7-8     | 7-2  | 3-9*  |      |      |
| G        | 7-5*  | 7-8     | 7-2* |      |      |      |
| H        | 6-6*  | 5-8*    | 5-5* |      |      |      |
| I        | 5-2*  | 5-1*    | 5-4  |      |      |      |
| J        | 5-2*  | 4-8     | 5-4  |      |      |      |
| K        | 3-9*  | 3-8*    | 4-9* |      |      |      |
| L        | 2-4*  | 3-1*    | 4-6  |      |      |      |
| M        | 1-3*  | 3-0     | 2-8* |      |      |      |
| N        | 1-2*  | 2-2     | 2-3* |      |      |      |
| O        | 1-0*  | 1-7     | 2-3  |      |      |      |
| P        | 0-9*  | 1-5     | 1-9* |      |      |      |
| Q        | 0-8*  | 1-0*    | 1-5* |      |      |      |
| R        | 0-5*  | 0-7     | 1-5  |      |      |      |
| S        | 1-3   |         |      |       |      |      |

* Fragments cloned into plasmids.
separated DNA fragments were Southern-transferred onto a nitrocellulose filter, fixed at 80 °C, pre-hybridized, and hybridized with the probes of cloned plasmid DNA labelled with $^{32}$PdCTP (Maniatis et al., 1982). Hybridizations were carried out in 50% formamide at 42 °C for 6 to 16 h and the filters were washed with 0-1 × SSC at room temperature. Analysis of the hybridization data showed that most of the sequence of the BmNPV genome was unique (data not shown).

By comparing the overlapping areas of DNA for each restriction fragment a preliminary physical map was constructed. For further detailing of the map, the larger cloned DNA fragments were digested with two or more restriction enzymes and maps of the larger cloned fragments were also constructed. To confirm the location of these fragments, molecular sizes of the small fragments generated from the larger cloned fragments were compared with those of other cloned fragments or with original viral DNA by electrophoresis in agarose gels. By combining the various data obtained above, a detailed physical map was constructed for the six restriction endonucleases (Fig. 2). The zero-point of the physical map was established as one end of the PstI F fragment following the proposal of Vlak & Smith (1982). The zero-point was positioned here because the polyhedrin gene (indicated by the arrowhead in Fig. 2) has been mapped in this fragment (Maeda et al., 1985).

From the hybridization experiments, five regions containing homologous sequences with several EcoRI sites were also found in the genome. To confirm the existence of the repeated sequences, cloned or subcloned plasmids [HindIII H, PstI K, HindIII–PstII (67.7 to 75.3 map units) of PstI B; KpnI D, and PstI–HindIII (96.1 to 99.6 map units) of HindIII] were digested with EcoRI and analysed by electrophoresis. As expected, several EcoRI fragments less than 400 bp were identified on a 1.5% agarose gel (data not shown). These areas containing several smaller EcoRI fragments seemed to be the so-called repeated sequences also found in AcNPV (Erlandsen et al., 1984; Guarino et al., 1986) and other baculoviruses (Kuzio & Faulkner, 1984; Arif & Doerfler, 1984). Three repeated sequences could not be mapped exactly; however, their locations are at either end or on both ends of the small EcoRI N, P, and R fragments. These areas were mapped and are indicated by the bars in Fig. 2. All five repeated sequences of the BmNPV

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**Fig. 2.** Physical map of BmNPV DNA for EcoRI, HindIII, PstI, BamHI, KpnI and SmaI. The circular DNA is presented in linear form. Map units are calculated assuming the total length of the genome is 100. The arrowhead indicates the position and direction of the polyhedrin gene. Bars represent areas of repeated sequences. Letters correspond to fragments in Fig. 1.
genome were located at positions similar to those of the repeated sequences mapped in the AcNPV genome (Summers & Smith, 1987). Furthermore, the positions of seven [3-4, 28-1, 38-9, 64-4 (KpnI), 64-4 (SmaI), 77-5 and 80-6 map units] of 14 restriction sites for BamHI, KpnI and SmaI were located very close to the analogous sites mapped in the AcNPV genome. This is consistent with data showing about 80% DNA homology of the polyhedrin gene area between BmNPV and AcNPV (Maeda et al., 1985; Iatrou et al., 1985). DNA hybridization analysis also indicated that the BmNPV genome has more than 50% homology with the AcNPV genome by calculation from the intensity of the exposed film when the viral DNAs were used for hybridization in 50% formamide at 42°C (S. Maeda et al., 1989).

There are reports that the genome organizations of NPVs are relatively conserved even between viral DNAs with low DNA homology (Leisy et al., 1984). From our results AcNPV and BmNPV seem to be closely related viruses in terms of sequence homology. This is of interest because of the significant differences in the phenotypic and biological characteristics of the two viruses, such as host range. We are now analysing the viral genome based on the physical map we constructed. The physical map and gene library will be useful for the further genetic studies of BmNPV.

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