Conserved Homologous Regions between Two Baculovirus DNAs

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SUMMARY

Regions of homology on the physical maps of Spodoptera exempta multiple-nucleocapsid nuclear polyhedrosis virus (SeMNPV-25), an Autographa californica MNPV genomic variant, and S. frugiperda (SfMNPV-2) baculovirus DNAs were identified by reciprocal DNA–DNA blot hybridization under conditions of an effective temperature of $T_m - 25^\circ C$. In addition, cloned fragments of the viral genome which contained the homologous regions were used in hybridization experiments to confirm, refine and correlate the regions of the two physical maps. Five homologous regions conserved between the two physical maps were identified. When the stringency of the hybridization was increased ($T_m - 20^\circ C$), only two of the original regions were identified by blot hybridization. One of the two regions contained the polyhedrin gene, and the other region was not associated with any known viral function. The five regions did not overlap with the intragenic homologous sequence (hr1 to hr5) regions on the SeMNPV-25 map or with restriction endonuclease variant (vI to vIV) regions on the SeMNPV-25 and SfMNPV-2 maps. The degree of similarity in the genomic organization of these two baculoviruses is discussed.
21AE cells maintained in 150 cm² flasks in BML/TC-10 medium (Knudson & Tinsley, 1974; Knudson & Buckley, 1977). Extracellular virus and viral DNA were purified as described previously (Maruniak et al., 1984). Purified viral DNA was restricted with enzymes, and the digested DNA samples were electrophoresed through a 0.75% (w/v) agarose gel using the procedures described previously (Maruniak et al., 1984).

Methods for bidirectional DNA transfer from agarose gel to the nitrocellulose paper are described elsewhere (Maruniak et al., 1984). The nitrocellulose blot was prehybridized, hybridized and washed as described previously (Maruniak et al., 1984). The hybridization buffer contained 40% (v/v) formamide and 4 × SSC or 50% (v/v) formamide and 5 × SSC with effective temperatures of hybridization calculated as $T_m = -25^\circ C$ or $T_m = -20^\circ C$, respectively (Howley et al., 1979) using G + C values of 43% for SeMNPV-25 and 44.7% for SfMNPV-2 (Knudson & Tinsley, 1978).
A blot containing SeMNPV-25 DNA restricted with enzymes was hybridized with labelled genomic SfMNPV-2 DNA (Fig. 1a). SfMNPV-2 hybridized to five regions of the SeMNPV-25 genome, and the regions defined by these data were located on the SeMNPV-25 map. Likewise, a blot containing SfMNPV-2 DNA restricted with enzymes was hybridized with labelled SeMNPV-25 DNA (Fig. 1b). SeMNPV-25 hybridized clearly to four regions on SfMNPV-2 with a possible fifth region, and the regions defined by these data were located on the SfMNPV-2 map. The variable fifth region was identified in the EcoRI-E fragment on the SfMNPV-2 map using the genomic probe SeMNPV-25. Nevertheless, its hybridization signal was variable. For example, a signal was observed in EcoRI-E (Fig. 1b, lane 7), but the corresponding region in BamHI-C (Fig. 1b, lane 1) exhibited a weak signal.

In order to localize the regions of cross-homology between the two baculoviruses, the genomes were cloned into pBR322 plasmids. Briefly, PstI-restricted viral DNA and pBR322 were prepared and ligated as described by Bolivar & Bachman (1979). The transformation of the ligated DNA into competent Escherichia coli HB101 cells has been described elsewhere (Cohen et al., 1972). Recombinant DNA plasmids were prepared for PstI digestion screening using a rapid alkaline extraction procedure (Birnboim & Doly, 1979). HB101 cells containing the recombinant pBR322 plasmids were grown in 1 l of L broth in spinner flasks at 37 °C. The plasmids were amplified by the addition of chloramphenicol (170 μg/ml) and grown overnight. The plasmid DNAs were purified following a modification of the procedure described by Godson & Vapnek (1973) and by equilibrium centrifugation in preformed CsCl–ethidium bromide (EtBr) gradients. The bands were collected, the EtBr was removed, and the DNA was dialysed against TE buffer (10 mM-Tris-HCl pH 8.0, 0.1 mM-EDTA).

A blot of PstI-restricted plasmids which contained PstI fragments of SeMNPV-25 was hybridized using SfMNPV-2 genome as a probe. SfMNPV-2 DNA hybridized to SeMNPV-25 plasmids containing fragments PstI-A, PstI-D, PstI-E, PstI-H and PstI-K (data not shown). The PstI-K fragment represented the variable fifth region mentioned above. When a blot containing the cloned PstI DNA fragments of SfMNPV-2 was hybridized with SeMNPV-25 genome, SeMNPV-25 DNA hybridized to SfMNPV-2 plasmids containing fragments PstI-B, PstI-D and PstI-I (data not shown).

The cloned viral fragments containing the conserved homologous regions were isolated from the plasmid DNA. The plasmids were restricted with PstI, nick-translated, and electrophoresed through a 0-83% low melting point agarose gel (SeaPlaque, Marine Colloids, Rockland, Me., U.S.A.). The cloned viral PstI fragment was isolated from the agarose as described previously (Maruniak et al., 1984). The nick-translated fragments were used in hybridization experiments to correlate the homologous regions between the two physical maps. Blots containing SeMNPV-25 DNA restricted with enzymes were hybridized with SfMNPV-2 cloned fragments PstI-D and PstI-I. The genomic SfMNPV-2 probe identified a 1-4 kbp Sfhrl region at 27-8 to 28.8 map units (m.u.), a 3.4 kbp Sfhr3 region at 29-9 to 32-5 m.u. and a 5-2 kbp Sfhr4 region at 37-2 to 41-2 m.u. on the SeMNPV-25 physical map (Fig. 2). SfMNPV-2 PstI-A and PstI-B fragments were not used as probes, because they would not further define these regions (Sfhr2 to Sfhr4) of homology. SfMNPV-2 PstI-I and PstI-D hybridized to a 0-8 kbp (Sfhr1) region at 2-9 to 3-5 m.u. and a 2-1 kbp (Sfhr5) region at 48-4 to 50-0 m.u. on the physical map of SeMNPV-25, respectively (Fig. 2).

Blots containing SfMNPV-2 DNA restricted with enzymes were hybridized with SeMNPV-25 cloned viral fragments PstI-D, PstI-E, PstI-H and PstI-K. SeMNPV-25 PstI-A was not used as a probe, because it would not further delineate the region (Sehr5) of homology. SeMNPV-25 PstI-D hybridized to a 1-4 kbp (Sehr1) region at 0-7 to 1-9 m.u. on the SfMNPV-2 physical map. SeMNPV-25 PstI-E hybridized to a 2-1 kbp (Sehr3) region at 21-3 to 23-9 m.u. on the SfMNPV-2 physical map. SeMNPV-25 PstI-H hybridized to a 5-8 kbp (Sehr4) region at 32-6 to 37-4 m.u. on the SfMNPV-2 map (Fig. 1c). SeMNPV-25 PstI-K hybridized to a 1-5 kbp (Sehr2) region at 64-1 to 65-9 m.u. on the physical map of SfMNPV-2 (Fig. 2).

The data from these experiments with isolated fragments further refined the regions of homology and confirmed their location on the physical maps of the two viruses (Fig. 2). Under these hybridization conditions (Tm -25 °C), sequences with approximately 18% mismatch would form stable hybrids. When hybridization stringency was increased to 14% base mismatch...
Fig. 2. Restriction endonuclease and summary genomic organization maps of SeMNPV-25 and SiMNPV-2 baculoviruses with the circular genomes presented in a linear form with map units. The restriction maps of SeMNPV-25 (Brown et al., 1984) and SiMNPV-2 (Maruniak et al., 1984) have been reported previously. On each series of restriction endonuclease maps, the regions that hybridize with a given probe are depicted as boxes (see key at right-hand side). The two summary maps of the genomic organization depict the conserved regions (Se/Sfhr1 to 5) of homology between the genomes, the intragenic homologous regions (hr1 to hr5) of SeMNPV-25 (Brown et al., 1984, 1985) following the convention of Coehran & Faulkner (1983), and the variant regions (vI to IV) as described previously (Brown et al., 1985; Maruniak et al., 1984) (see key). The double-headed arrows indicate the Se/Sfhr regions that are cross-homologous. 

(T_m - 20 °C), only regions Sfhr1 and Sfhr5 were observed when the SeMNPV-25 genome was probed with SiMNPV-2 DNA (data not shown).

A number of studies of homology (Jewell & Miller, 1980; Rohrmann et al., 1982; Smith & Summers, 1982) have used the blot hybridization procedure to study the genetic relatedness of baculoviruses. Although these studies used hybridization conditions different from those used in
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this study, homologous regions were identified by Jewell & Miller (1980) and by Smith & Summers (1982) which corresponded to the homology regions identified between SeMNPV-25 and SfMNPV-2. Both studies found a highly conserved region in the baculovirus genome which corresponds to SeMNPV-25 homology region Sfhrl. Region Sfhrl is located within SeMNPV-25 EcoRI-I, and it contains the gene that encodes polyhedrin (Summers et al., 1980; Vlak et al., 1981; Brown et al., 1984). Since the polyhedrin and granulin proteins of the viruses in the study of homology by Smith & Summers (1982) have similar antigenic determinants (Smith & Summers, 1981) and tryptic peptides (Maruniak & Summers, 1978), it is not surprising that the area of the genome coding for the polyhedrin is highly conserved. Smith & Summers (1982) described two additional regions of homology which coincided with SeMNPV-25 homology regions, Sfihr2 and Sfihr3. Region Se/Sfihr2 was conserved in a number of MNPV and granulosis viruses (GV) baculoviruses, whereas region Se/Sfihr3 was conserved in MNPV, SNPV and GV baculoviruses. Jewell & Miller (1980) and Smith & Summers (1982) noted other regions of weaker homology elsewhere on the baculovirus genome, but these locations were not provided. Whether these regions overlap with SeMNPV-25 homology regions Sfihr4 and Sfihr5 remains to be demonstrated. Both studies found homology regions in addition to those described here between SeMNPV-25 and SfMNPV-2. The discrepancies between their results and the data presented here may be due to their less stringent hybridization conditions or to actual differences in SfMNPV isolates which were used. One homology region described by Smith & Summers (1982) codes for a late 10K viral gene product (Smith et al., 1982). SfMNPV-2 did not hybridize to the region of SeMNPV-25 that may encode the 10K protein. However, only three of the nine MNPVs Smith & Summers (1982) reported shared sequence homology with AcMNPV in this region, and the function of the 10K is not known.

The five homologous regions of SeMNPV-25 and SfMNPV-2 did not overlap with the variant regions in SeMNPV-25 (Brown et al., 1985) or in SfMNPV-2 (Maruniak et al., 1984), the SeMNPV-25 intragenic homologous regions (hr1 to hr5) (Brown et al., 1984, 1985), or with the variant regions reported in the literature for AcMNPV as summarized recently (Brown et al., 1985). Thus, the Se/Sfihr1 to Sfihr5 regions may contain DNA sequences that encode common essential viral functions.

The SeMNPV-25 and SfMNPV-2 genomes have a similar organization. The Se/Sfihr1, 3, 4 and 5 regions exhibited a similar spatial relationship on the physical maps of SeMNPV-25 and SfMNPV-2 (Fig. 2). Region Sehr2 was the only exception, because it was located approximately 180° to the other side of the genome in SfMNPV-2 when compared with Sfihr2 in SeMNPV-25. Conservation of the genome organization between OpMNPV and AMNPV has been suggested. However, the transcription of the polyhedrin genes of AcMNPV and OpMNPV were in the opposite direction, indicating a genomic inversion relative to AcMNPV (Leisy et al., 1984).

Thus, the two baculovirus genomes contain five conserved homologous regions. One highly conserved region encodes the polyhedrin. Although the other highly conserved region has not been associated with any specific viral function, transcriptional mapping data suggest that region Sfihr5 is near an area which is transcribed early in infection (Lubbert & Doerfler, 1984). The conservation of genetic information between these baculoviruses may suggest regions on the genomes which encode essential functions. Clearly, these regions warrant further fine-scale analyses.

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REFERENCES


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