Short communication

Physical map of Anagraphe falcifera multinucleocapsid nuclear polyhedrosis virus

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A physical map of Anagraphe falcifera multinucleocapsid nuclear polyhedrosis virus (AfMNPV) DNA was constructed for restriction endonucleases EcoRI, HindIII, PstI and XhoI. The genome size was estimated to be 130 kbp. Ordering of the restriction fragments was accomplished by cross-blot hybridization, double digestion and DNA–DNA hybridization. The polyhedrin gene and homologous repeat (hr) regions were located by hybridization to the Autographa californica MNPV (AcMNPV) polyhedrin gene and hr4, respectively. Restriction pattern comparison and Southern blot analysis suggest that AfMNPV is closely related to AcMNPV.

Baculoviruses are invertebrate-specific pathogens that are potential biological agents for controlling insects. Most baculovirus infections are limited to a single host species or at most a few closely related species (Gröner, 1986). Autographa californica multinucleocapsid nuclear polyhedrosis virus (AcMNPV), the best studied baculovirus, reportedly infects 33 species from 10 families of Lepidoptera (Gröner, 1986). Among these, Agrotis segetum, Helicoverpa zea (Heliothis zea) and Mamestra brassicae were more recently classified as non-permissive species for AcMNPV (Bishop et al., 1988). The reason for this discrepancy is unknown. It could be due to differences between geographical strains of insects. However, because the report by Gröner (1986) is a compilation of earlier studies, many conducted before sensitive techniques for unambiguous virus identification were available or widely used, the more recent study most likely reflects the true AcMNPV host range. Anagraphe falcifera MNPV (AfMNPV), originally isolated from the celery looper, A. falcifera, is also reported to be infectious to a wide range of economically important insect pests (Hostetter & Puttler, 1991). More than 31 species from 10 families of Lepidoptera were susceptible to AfMNPV infection (Hostetter & Puttler, 1991). Four of these species, Peridroma saucia, Pieris rapae, Manduca sexta and Plutella xylostella, are not susceptible to AcMNPV (Bishop et al., 1988). Dose–mortality comparisons of AfMNPV with AcMNPV demonstrate that AfMNPV has a 30-fold lower LC50 against H. zea (Hostetter & Puttler, 1991), a major pest of corn and cotton that has developed resistance to many chemical insecticides (Abd-Elghafar et al., 1993). Thus, AfMNPV is a potential microbial insecticide for H. zea. AfMNPV also infects the navel orange worm, Amyelois transitella, a major pest of almonds (Hostetter & Puttler, 1991; Vail et al., 1993). It is the first baculovirus patented by the US Government Patent and Trademark Office (Hostetter & Puttler, 1991).

Reports of over 600 insect species infected by baculoviruses were catalogued by Martignoni & Iwai (1986), but few of these viruses have been isolated or characterized. Although some baculoviruses have been studied at the molecular level (reviewed by O’Reilly et al., 1992), relatively little is known about the mechanisms that determine baculovirus host range. A 572 bp region of the DNA helicase gene from Bombyx mori NPV (BmNPV), with only 29 nucleotide differences from the AcMNPV helicase gene, enabled a hybrid AcMNPV to replicate in a B. mori cell line (Maeda et al., 1993). p35, an AcMNPV gene responsible for blocking the apoptotic response (Clem et al., 1991), is also involved in host range determination. The infectivity of p35 deletion mutants is reduced in Spodoptera frugiperda but not Trichoplusia ni larvae when budded virus is injected (Clem & Miller, 1993) or when occluded virus is administered per os (Clem et al., 1994).

By comparing AfMNPV with other baculoviruses it may be possible to identify additional genes or regions of
Fig. 1. (a) Gel photograph and (b) schematic representation of restriction fragment profiles of AfMNPV (lane 1) and AcMNPV (lane 2) DNA cleaved with EcoRI, HindIII, PstI and XhoI. Restriction fragment designations are indicated by letters in the schematic drawings of individual restriction endonuclease profiles. Arrows on the diagram indicate co-migrating AfMNPV and AcMNPV restriction fragments. The sizes of phage \( \lambda \) restriction fragments size standards are shown in kbp.

AfMNPV was propagated in third instar \( T. \) ni larvae. Haemolymph was collected and used to inoculate \( S. \) frugiperda Sf21 cells. A plaque representing the most prevalent restriction endonuclease pattern was used to make a working stock. Virus DNA was purified from the budded virus (O'Reilly et al., 1992), digested with endonucleases EcoRI, HindIII, PstI or XhoI (Boehringer Mannheim) and separated by electrophoresis on a 0.7% agarose gel (Fig. 1). The sizes were estimated according to the migration distances on the gels compared with the known sizes of phage \( \lambda \) HindIII fragments and various restriction fragments of AcMNPV used as size standards. For fragments with sizes larger than 15 kbp a second enzyme was used to generate smaller fragments, providing a better estimate. The enzymes EcoRI, HindIII, PstI and XhoI cleaved the genome into 22, 21, 13 and 14 fragments, respectively, with sizes ranging from 31 kbp to 0.5 kbp (Table 1). These numbers represent the minimum number of cleavage sites for each of these four enzymes, since fragments smaller than 0.5 kbp are not detected. The total genome size of AfMNPV was estimated to be 130 kbp.

To detect collinearity between different restriction fragments, the cross-blot hybridization technique was used (Potter & Dressler, 1986). For the 'hot blot', AfMNPV HindIII fragments were \( \gamma ^{32} \)P-end-labelled (\( \gamma ^{32} \)P]ATP; 3000 Ci/mmol; DuPont NEN) using T4 kinase (Sambrook et al., 1989). Cold blots comprised EcoRI, HindIII, PstI and XhoI restriction fragments. Restriction fragments were resolved on agarose gels and transferred to GeneScreen nylon membranes (Biotechnology Systems). Hybridization was at 42 °C overnight. By determining the collinearities between fragments generated by different restriction endonucleases, a preliminary AfMNPV physical map was developed.
Table 1. AfMNPV DNA restriction endonuclease fragment sizes in kbp

<table>
<thead>
<tr>
<th>Fragment</th>
<th>EcoRI</th>
<th>HindIII</th>
<th>PstI</th>
<th>XhoI</th>
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<td>17.0</td>
<td>15.1</td>
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<tr>
<td>V</td>
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<td>130.0</td>
<td>129.1</td>
<td>129.6</td>
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</table>

AfMNPV physical map

Partially digested AfMNPV HindIII fragments were cloned into a cosmide, pVK102 (Knauf & Nester, 1982), to generate an overlapping genomic library. The locations of fragments whose positions were uncertain were established by double digestion experiments and Southern blot analysis using cloned fragments from the AfMNPV library as probes. Fragments were 32P-labelled ([32P]dCTP; 3000 Ci/mmol; DuPont NEN) by nick translation (Sambrook et al., 1989). Hybridizations were performed in 50% formamide hybridization buffer at 42°C (Sambrook et al., 1989). Double digestions were carried out with reciprocal enzyme pairs of EcoRI, HindIII, PstI and XhoI.

A linear physical map of AfMNPV is shown in Fig. 2. To define the zero point of the AfMNPV map (Vlak & Smith, 1982), Southern blots of AfMNPV restriction fragments were probed with the AcMNPV polyhedrin gene. The probe was an EcoRV–DraI fragment (4427–5424 kbp; Ayres et al., 1994) of AcMNPV DNA. The probe hybridized to EcoRI G, HindIII Q, PstI E and XhoI C. These fragments were located to the left of the map; the left end of the smallest fragment, HindIII Q, was assigned as 0 m.u. (Vlak & Smith, 1982). The map orientation was based on collinearity with the AcMNPV physical map. All of the co-migrating restriction fragments (Fig. 1) from each virus cross-hybridized except for the HindIII P fragments. AfMNPV EcoRI D, F, J, M and R, HindIII A, J, O, R and T, PstI F, I, J and L and XhoI A, B, E, F, K, L and M fragments cross-hybridized with AcMNPV EcoRI D, G, J, M and Q, HindIII A, I, O, S and U, PstI G, H, I and O and XhoI A, B, E, F, K, L and M, respectively (data not shown). These cross-hybridizing restriction fragments were distributed in the same order across both the AfMNPV and AcMNPV physical maps, demonstrating the collinearity of the virus genomes.

Homologous regions (hr) in AcMNPV function as enhancers of early mRNA transcription in transient assays (Guarino & Summers, 1986) and as origins of virus DNA replication (Kool et al., 1993; Leisy & Rohrmann, 1993). Eight hrs were located in AcMNPV; these regions have two to eight reiterations of an imperfect palindromic sequence containing an EcoRI site at the centre of each palindrome (Ayres et al., 1994). The AcMNPV EcoRI Q fragment, containing the hr4L region, was 32P-labelled by random priming (Sambrook et al., 1989) and used for Southern blot analysis. This fragment hybridized to AfMNPV HindIII C, H, D, E and M, PstI F, G, A, C and B and XhoI C, I, A, B and K fragments, but to only one EcoRI fragment, EcoRI R, indicating that there are EcoRI site-containing hr regions in AfMNPV. Based on the overlapping regions and the corresponding EcoRI sites, we located a minimum of five hrs that were homologous to AcMNPV hr4, one each at the left sides of EcoRI J, F and N and on either the right or left sides, or both sides, of the EcoRI Q and R restriction fragments of AfMNPV (Fig. 2a). These hr regions are in similar positions to the hr regions in AcMNPV (Fig. 2b).

A comparison of the physical maps of AfMNPV and AcMNPV indicates that the arrangement of restriction fragments of these two viruses is similar, especially for XhoI. Although they have different numbers of XhoI restriction fragments (14 for AfMNPV and 13 for AcMNPV), seven co-migrate (Fig. 1b). All co-migrating restriction fragments are in similar positions in both viruses and five of them, XhoI E, L, M, A and B, form a contiguous array (Fig. 2b). Although the other three AfMNPV restriction patterns examined are different from those of AcMNPV, we noted a number of similarities. For each of the other restriction enzymes, all of the co-migrating restriction fragments, except HindIII P, were located in the same positions in each virus. Several also comprised contiguous arrays. These include AfMNPV EcoRI F, D and R, HindIII O, T, J and A and PstI F, L and J and their corresponding AcMNPV restriction fragments (EcoRI G, D and Q, HindIII O, U, I and A and PstI G, O and L). These arrays of restriction fragments, which are the same in both AcMNPV and AfMNPV, overlap and span the AfMNPV physical map between 6 and 94.2 kbp. The remainder of the AfMNPV physical map differs more from the AcMNPV physical map. Only one restriction fragment that co-migrates with an AcMNPV fragment, XhoI K, is located in this region.
Fig. 2. (a) Physical map of AfMNPV. Restriction enzymes are indicated to the right of the map. Vertical lines indicate cleavage sites and the numbers below each restriction site indicate the distance in kbp from the left end. The HindIII Q fragment is the smallest restriction fragment to hybridize with the AcMNPV polyhedrin gene and by convention is designated as the left end of the linearized, circular genome. The hrs are shown as bold lines. hr1 and hr4, marked with asterisks, could be on either the right or left sides, or both sides of EcoRI Q and R, respectively. A scale indicating m.u. and kbp is shown at the bottom. EcoRI U and V fragments are not indicated on the map. (b) Comparison of XhoI restriction fragment maps of AfMNPV and AcMNPV. The AcMNPV map has been shifted to the left to align it with the AfMNPV map. The left end of the AcMNPV XhoI map corresponds to 4.5 kbp. The locations of polyhedrin (polh) genes are indicated by bars. The locations of AcMNPV hr regions are indicated by '#' below the AcMNPV map. (Fig. 2b). While they do not co-migrate on gels, the AfMNPV restriction fragments within this region hybridize to AfMNPV restriction fragments located in similar positions on the AcMNPV physical map (data not shown). Collinear physical maps, similar XhoI restriction patterns, identical arrays of contiguous restriction fragments for each restriction map, EcoRI-containing hr regions and equivalent genome sizes, as well as the broad larval host range and in vitro infectivity (Hostetter & Puttler, 1991; McIntosh, 1991; Vail et al., 1993) suggest that AfMNPV and AcMNPV are related viruses.

Despite the similarities between the AfMNPV and AcMNPV physical maps their larval host ranges differ. AfMNPV infects some insects that are not susceptible to AcMNPV, such as A. transitella. Although both viruses can infect some of the same hosts their infectivity varies. For example, AfMNPV is more infectious for H. zea than AcMNPV. Comparative studies of these viruses may help to determine the underlying mechanisms for these differences in infectivity.

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References


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