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Orientation of the Genome of *Autographa californica* Nuclear Polyhedrosis Virus: a Proposal†‡§

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The nuclear polyhedrosis virus of the alfalfa looper *Autographa californica* contains a double-stranded, circular DNA genome. Fourteen scientists agreed to accept an orientation of this circular genome with respect to a physical map of the restriction endonuclease cleavage sites.

Nuclear polyhedrosis viruses (NPVs) are subgroup A baculoviruses. The rod-shaped nucleocapsids are enveloped either singly (SNPV) or in bundles (MNPV) and are occluded in a large protein crystal, the occlusion body. The virus replicates exclusively in the insect cell nucleus. NPVs have been reported from over 300 sources. The genome of NPVs consists of a double-stranded, circular DNA molecule ranging in size from 50×10^6 to over 100×10^6 daltons (4).

The NPV of the alfalfa looper *Autographa californica* has gained the interest of many researchers since this virus has the potential to be a biological control agent for many Lepidopteran pests. The multiply embedded type of this virus (AcMNPV) was adopted as the prototype of subgroup A baculoviruses by the International Committee on Taxonomy of Viruses in 1979. There are several plaque-purified variants from wild isolates (3, 5, 8, 11, 15; M. A. Cochran, E. B. Carstens, B. T. Eaton, and P. Faulkner, J. Virol., in press), which are closely related and differ in only a few restriction endonuclease fragments. A well-characterized example of AcMNPV is the E2 variant (8) for which the DNA structure has been described in detail (9, 13).

In recent years extensive studies have been performed on the structure and multiplication of AcMNPV. Physical maps of the DNA have been constructed using the restriction endonucleases *Eco*RI, *Bam*HI, *Sma*I, *Hin*dIII, *Kpn*I, *Sac*I, *Xho*I, and *Pst*I (7, 9, 12; Cochran et al., in press). However, the orientation of the fragments and the zero point on the circular DNA have been chosen arbitrarily. Mutants of AcMNPV have been obtained (1, 6, 15), and genetic maps are being constructed using classical genetic recombination studies (2) and marker rescue experiments (7a).

To overcome the present inconvenience and to avoid future confusion as a result of different genome orientations, we felt that it was necessary to choose a convention for the orientation of physical maps and location of a zero point on the circular genome of AcMNPV, the most widely studied baculovirus. The number of physical or biological markers on the genome to serve as points of reference is very limited. Restriction endonucleases generating only one cut in the circular molecule, as is the case in many animal and bacterial virus DNAs, have not been found for AcMNPV. By following the segregation of genes in recombination studies, the gene for the occlusion body protein (= polyhedrin) was mapped in a region of the genome that included the *Eco*RI-I fragment (10). The polyhedrin gene has recently been located on the *Eco*RI-I fragment by in vitro translation of polyhedrin from *Eco*RI-I-specific RNA (14). This fragment could serve as one point of reference. A second point of reference could be map position 0.73 between *Eco*RI-E and *Eco*RI-L, since several AcMNPV variants (M3 and S3) and a related virus, *Galleria mellonella* MNPV (GmMNPV), have a small insertion at this position on the genome (9).

By generally accepted convention, the genetic and physical maps of AcMNPV DNA will be drawn (Fig. 1 and 2) with the following asymmetric and biological features defining the orien-

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§ Specimens of AcMNPV strain E2 can be obtained from Max D. Summers, Department of Entomology, Texas A&M University, College Station, TX 77843.

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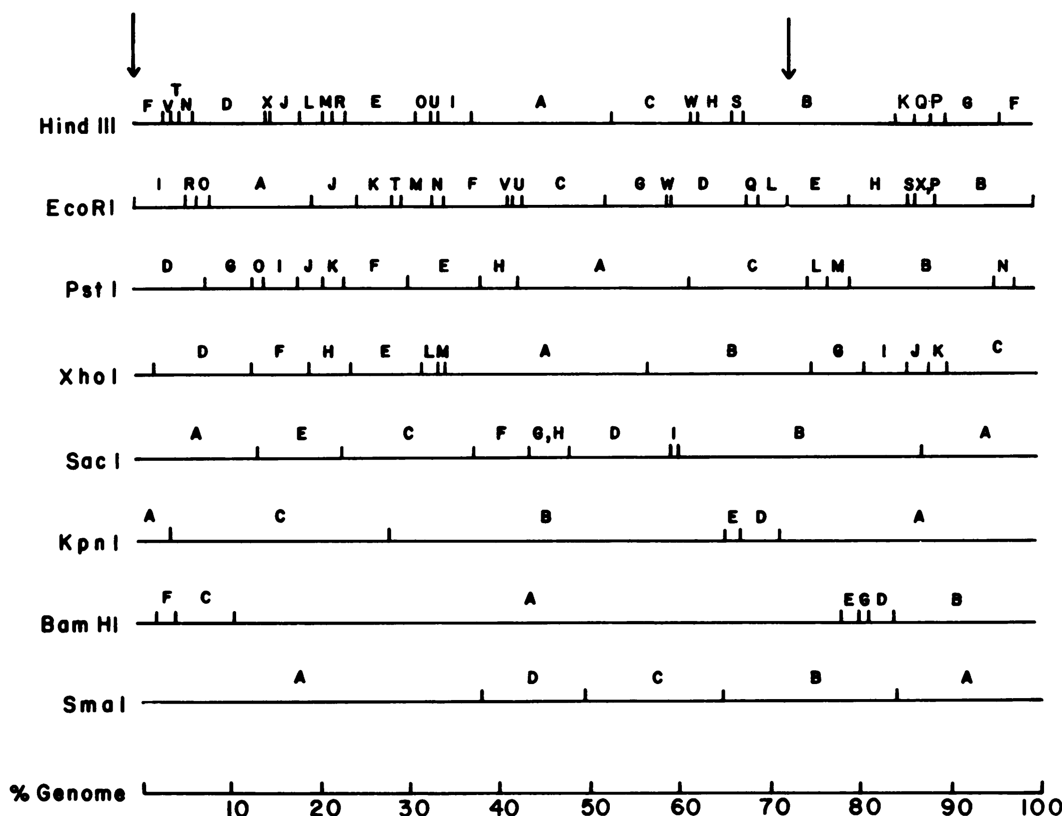

FIG. 1. Physical map of AcMNPV DNA (variant E2) for the restriction endonucleases *Sma*I, *Kpn*I, *Bam*HI, *Sac*I, *Xho*I, *Eco*RI, *Hin*dIII, and *Pst*I. The circular DNA (81.9×10^6 daltons) is presented in a linear form. The distance on the physical map is expressed as a percentage of the genome. The *Eco*RI cleavage sites between fragments B and I and fragments E and L were chosen as the zero point and at about 73% (arrows) of the genome, respectively, to fixate the location of the fragments.

tation of the genome. (i) Restriction fragments are lettered alphabetically starting with A for the fragment with the lowest mobility in agarose gels. (ii) Capital letters (A, B, C, . . .) followed by small letters (. . . Y, Z, a, b, c, . . .) are used to designate restriction fragments. (iii) Comigrating restriction fragments are given multiple-letter designations up to the number of fragments contained (e.g., *Eco*RI-FGH). (iv) Adjacent fragments, the order of which on the physical map has not been determined, are indicated by double lettering (e.g., *Eco*RI-U,V and *Sac*I-G,H). (v) The zero point of reference is positioned between *Eco*RI-I and *Eco*RI-B. (vi) The position on the genome having the insertions in the M3 and S3 variants and GmMNPV is at about 0.73 map unit. (vii) The order of the *Eco*RI fragments is running clockwise I, R, O, A, . . . to *Eco*RI-B. The order of fragments from other restriction endonucleases is correlated with this *Eco*RI map (Fig. 1 and 2).

Transcription studies are under way in several laboratories. To avoid the cumbersome notation of designating a particular strand after the physical property employed to separate the DNA chains, we recommend the acceptance of the nomenclature developed in bacteriophage systems: (viii) on the linearized AcMNPV DNA map (Fig. 3), the DNA strand transcribed from right to left should be denoted the R-strand, and its complement, which is transcribed from left to right, should be denoted the L-strand.

When the genome of AcMPNV has been oriented according to this proposal, the positions on the map should be defined as the percentage of the total distance of the genome as indicated in Fig. 1. Genetic, physical, transcription, and functional maps of AcMNPV DNA and its variants should be oriented in agreement with the present recommendation to facilitate direct comparisons of information from different laboratories.

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FIG. 2. Circular map of AcMNPV DNA (variant E2). See legend to Fig. 1.

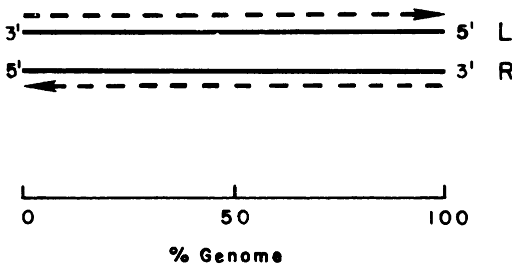

FIG. 3. DNA strand nomenclature of the linearized genome of AcMNPV and direction of transcription (– – →).

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