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The *Lymantria dispar* Nucleopolyhedrovirus Contains the Capsid-Associated p24 Protein Gene

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Abstract. During the course of investigations on a wild-type strain of *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus (LdMNPV), a region of the viral genome was analyzed and found to contain 697 bp that is lacking in the sequenced strain (5–6) of LdMNPV (Kuzio et al., Virology *253*, 17–34, 1999). The sequenced strain of LdMNPV contains a mutation in the *25 K few polyhedra* (FP) gene, and exhibits the phenotype of a FP mutant. The additional sequence was located at approximately 81.4 map units within the viral genome, and was found in 10 different wild-type LdMNPV genotypic variants analyzed. Since the additional sequence was found in all wild-type virus strains analyzed, this sequence should be included in the representative LdMNPV genome. Sequence analysis of the genomic region containing the additional sequences revealed the presence of a homologue of the *Autographa californica* MNPV capsid-associated *p24* gene (ORF 129). This gene, absent in LdMNPV isolate 5–6, is also present in the *Orgyia pseudotsugata* MNPV, *Bombyx mori* NPV, *Spodoptera exigua* MNPV, *S. litura* MNPV, *Mamestra configurata* MNPV, *Helicoverpa armigera* SNPV, *H. zea* SNPV, *Buzura suppressaria* SNPV, *Xestia c-nigrum granulovirus*, *Plutella xylostella* GV, and *Cydia pomonella* GV.

Key words: *Autographa californica* nucleopolyhedrovirus, baculovirus, capsid-associated p24 gene, *Lymantria dispar*, *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus

The Baculoviridae are a group of viruses that contain circular double-stranded DNA genomes that infect over 300 insect species. Most baculoviruses identified to date infect lepidopteran insects, and exhibit a host range of one to over 30 species (see [1–4] for reviews). These viruses are being used to control insect pests in agriculture and forestry on a limited basis in the United States, and more extensively in South America and Asia. The *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus (LdMNPV) is registered by the Environmental Protection Agency as a gypsy moth, *L. dispar* (*L.*), control agent [5]. The gypsy moth is a serious defoliating insect pest in deciduous forests of the Northeastern United States and is spreading into the South and Midwest.

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As a consequence of its use as a gypsy moth control agent the LdMNPV has received considerable attention. Strains of LdMNPV have been developed that have improved gypsy moth control attributes [6], and cell culture production characteristics [7,8]. A number of LdMNPV genes have been characterized and the genome has been sequenced [9]. The LdMNPV isolate (5–6) that was sequenced is a few polyhedra (FP) mutant [10], and the mutation in this isolate was characterized [11]. Comparison of isolate 5–6 and wild-type LdMNPV genomic DNA restriction endonuclease digests revealed that isolate 5–6 contained a smaller Bg/ll fragment in the genomic region from approximately 127.5 to 138.0 kbp. Consequently, this genomic region of isolate 5–6 is not representative of wild-type LdMNPV. To provide a more accurate genomic sequence of wild-type LdMNPV the additional sequence present in wild-type LdMNPV was identified and characterized.

Budded virus or polyhedra were isolated from Ld652Y cells infected with LdMNPV isolate 5–6 [10], wild-type viral isolates A21, B21, 122, 163 [12], 111, 123, 141, 151, 201, and 203, and isolates A21-MPV and 51f as previously described [13], and used as a source of genomic DNA for restriction analysis. The LdMNPV wild-type isolates are genotypic variants, isolate 51f produces abnormal polyhedra (unpublished data), and isolate A21-MPV exhibits greater polyhedra production stability in Ld652Y cells during viral serial passage [7]. Viral DNA was digested with Bg/II and fractionated on a 0.7% agarose-tris-borate-EDTA (TBE) gel. *Bg/II* digestion of LdMNPV isolate 5–6 genomic DNA generated a Bg/II D fragment (from the genomic region of approximately 127.5 to 138.0 kbp) of approximately 10.5 kbp. In contrast, the *Bg/LL* D fragment was approximately 11.2 kbp in all other viral isolates examined (Fig. 1A).

To identify the additional sequence in wild-type LdMNPV strains, subclones of the *Bg/LL* D fragments from LdMNPV isolates 5–6 and A21-MPV were generated by standard techniques using the pBS vector (Stratagene, La Jolla, CA) and compared. Restriction fragment length polymorphisms between isolate 5–6 and A21-MPV clones were identified after restriction endonuclease digestion of genomic DNA and fractionation of fragments on 0.8% TBE gels. This analysis revealed that isolate A21-MPV contained an *Mfe*I/*Bam*HI fragment that was approximately 0.7 kbp larger than the *Mfe*I/*Bam*HI fragment from isolate 5–6 (depicted in Fig. 2A). Comparison of other restriction endonuclease digestion fragments from the *Bg/ll* D fragment from isolates A21-MPV and 5–6 revealed no differences (Fig. 2A).

The sequence of the viral genomic region (*Mfe*I/*Mfe*I fragment) containing the additional sequence was determined on both strands of pNPA21 MM using the dideoxynucleotide sequencing method. Plasmid templates were sequenced with the Sequitherm ExCell II sequencing kit (Epicenter Technologies) using the supplied protocol. [α-^{35} S] dATP was supplied by NEN. Sequence analysis was done using the MacVector program (IBI). Sequence analysis of this fragment revealed the presence of an additional 697 bp within the region bounded by the *Mfe*I (130.7 kbp on the 5–6 sequence) and *Bam*HI (131.6 kbp) sites (Fig. 2B). The additional sequence was located between the G nucleotides at positions 184 and 185 within the *Mfe*I/*Bam*HI fragment (Fig. 2B).

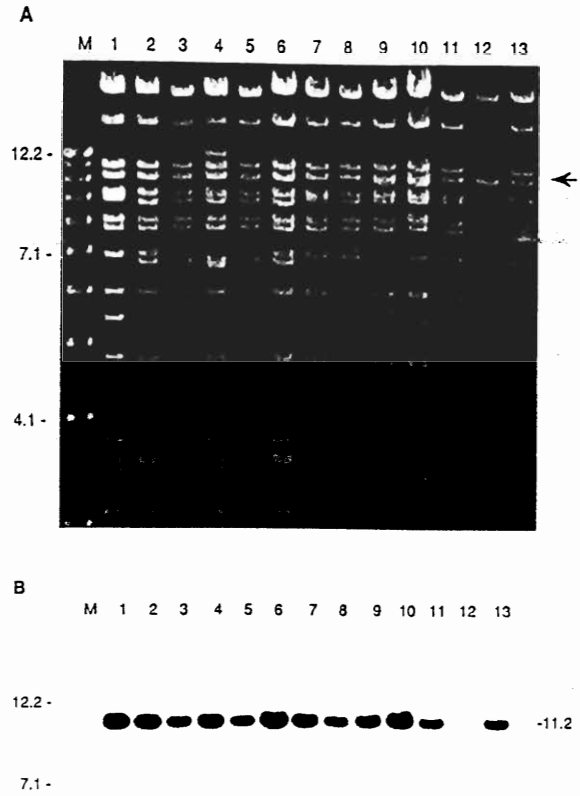

Fig. 1. Genotypic analysis of LdMNPV isolates. (A) Viral isolate genomic DNA was digested with the restriction enzyme *Bg*/II, and the fragments were separated on an agarose gel and visualized after staining with ethidium bromide. Lanes 1–13 contain DNA from viral isolates A21, B21, 111, 122, 123, 141, 151, 163, 201, 203, 51f, 5–6, and A21-MPV, respectively. The lane labeled M contains the DNA size markers, and the sizes of three of the markers are indicated on the left in kbp (Gibco BRL). The arrow on the right indicates the 11.2 kbp fragment present in all isolates except 5–6. (B) Hybridization of a *StuI*/*ScaI* fragment (see Fig. 2B) from isolate A21-MPV to a Southern blot of the gel shown in part A. The size of the fragment that hybridized with the probe is indicated on the right (in kbp).

The genomes of the LdMNPV genotypic variants analyzed in Fig. 1A were probed with the additional sequence identified in isolate A21-MPV to confirm its presence in these isolates. Southern blot analysis was performed on nytran membranes using a probe labeled with a Nick-Translation kit (Bethesda Research Laboratories) and [α-^{32} P]dCTP (NEN). The probe was generated by band-isolation of a *StuI*/*ScaI* fragment (containing only the additional sequence) from a *KpnI*-*XbaI* clone of isolate

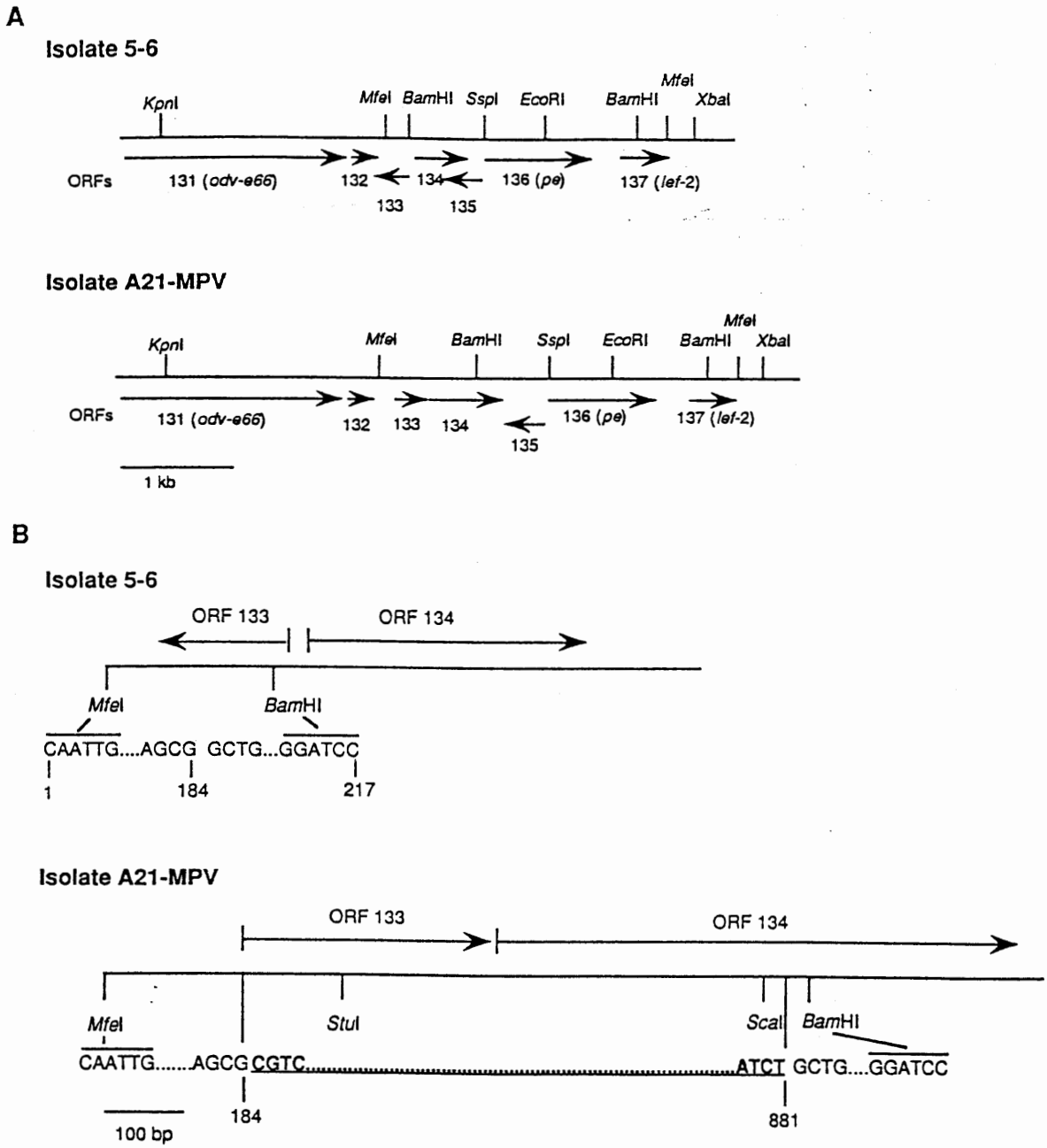

Fig. 2. Analysis of the genomic region containing the additional sequences found in isolate A21-MPV. (A) ORF and restriction endonuclease map of isolates 5–6 and A21-MPV of the region containing ORFs 131 through 137. (B) Location of the additional sequences found in isolate A21-MPV and of the resulting ORFs. The additional sequence is underlined.

A21-MPV (see Fig. 2B). The probe hybridized to a fragment of approximately 11.2 kbp in all of the viral isolates except isolate 5–6 (Fig. 1B). The probe did not hybridize to any fragment from the genome of isolate 5–6. This result confirms the presence of the additional sequence in the genotypic variants investigated, and indicates that this sequence is not present in another genomic location in isolate 5–6.

The additional nucleotides in isolate A21-MPV eliminates ORFs 133 and 134 in isolate 5–6, and generates new 133 and 134 ORFs. Another ORF is also generated; however, it is in a different reading frame from ORF 134 and is mostly contained within this ORF. The new ORF 133 in isolate A21-MPV is oriented in the 5′ to 3′ direction, and contains a TATA sequence 42 nucleotides upstream of the ATG and a CAGT mRNA start site sequence 28 nucleotides downstream from the TATA sequence (data not shown). Comparison of ORF 133 with the AcMNPV revealed no homologues. The new ORF 134 in A21-MPV is oriented in the 5′ to 3′ direction, and contains a late promoter sequence (TTAAG) 7 nucleotides upstream of the ATG (data not shown). The predicted amino acid sequence of ORF 134 was compared with AcMNPV proteins, and the protein encoded by AcMNPV ORF 129 (p24 capsid-associated protein [14]) was found to be homologous. The LdMNPV protein shows approximately a 42% amino acid identity to the AcMNPV protein, contains 223 amino acids, and has a predicted molecular weight of 24.0 kda (data not shown).

The *Orgyia pseudotsugata* MNPV (ORF 127 [15]), *Bombyx mori* NPV (ORF 106 [16]), *Spodoptera exigua* MNPV (ORF 10 [17]), *S. litura* MNPV (ORF 116 [18]), *Mamestra configurata* MNPV (ORF 12 [19]), *Helicoverpa armigera* SNPV (ORF 118 [20]), *H. zea* SNPV (ORF 122 [21]), *Buzura suppressaria* SNPV (ORF 11 [22]), *Xestia c-nigrum* granulovirus (ORF 80 [23]), *Plutella xylostella* GV (ORF 53 [24]), and *Cydia pomonella* GV (ORF 71 [25]), also contain homologues to ORF 134 of LdMNPV. The proteins encoded by these genes exhibit amino acid identities ranging from approximately 21–51%. Studies on the OpMNPV p24 protein has shown that this protein is found associated with both BV and ODV capsids [26]. Since the p24 protein encoded by LdMNPV ORF 134 is found in all wild-type viral isolates examined this ORF should be included in the representative LdMNPV virus strain. The deletion of ORF 134 from LdMNPV isolate 5–6 did not negatively impact BV formation or infectivity, or any other readily discernible viral process [10]. However, since isolate 5–6 is a FP mutant, it is possible that the FP phenotype is dominant over an ORF 134 null virus phenotype.

Nucleotide sequence accession number. The nucleotide sequence accession number of the sequence reported in this paper is AY214390.

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