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

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Received June 19, 2002; Accepted September 14, 2002

**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, *Bazura 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 [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.

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 BgII 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 polyheda 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 BglII and fractionated on a 0.7% agarose-tris-borate-EDTA (TBE) gel. BglII digestion of LdMNPV isolate 5–6 genomic DNA generated a BglII D fragment (from the genomic region of approximately 127.5 to 138.0 kbp) of approximately 10.5 kbp. In contrast, the BglII 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 BglII 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 MfeI/BamHI fragment that was approximately 0.7 kbp larger than the MfeI/BamHI fragment from isolate 5–6 (depicted in Fig. 2A). Comparison of other restriction endonuclease digestion fragments from the BglII D fragment from isolates A21-MPV and 5–6 revealed no differences (Fig. 2A).

The sequence of the viral genomic region (MfeI/MfeI 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. [γ-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 MfeI (130.7 kbp on the 5–6 sequence) and BamHI (131.6 kbp) sites (Fig. 2B). The additional sequence was located between the G nucleotides at positions 184 and 185 within the MfeI/BamHI fragment (Fig. 2B).

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 nitro membranes using a probe labeled with a Nick-Translation kit (Bethesda Research Laboratories) and [γ-32 P]dCTP (NEN). The probe was generated by band-isolation of a Stul/ScaI fragment (containing only the additional sequence) from a KpnI-XbaI clone of isolate