IDENTIFICATION, CLONING, AND EXPRESSION ANALYSIS OF THREE PUTATIVE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS IMMEDIATE EARLY GENES

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ABSTRACT

Viral immediate early gene products are usually regulatory proteins that control expression of other viral genes at the transcriptional level or are proteins that are part of the viral DNA replication complex. The identification and functional characterization of the immediate early gene products of *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) will further our understanding of viral pathogenesis at the molecular level and may yield insights into a molecular means of enhancing viral potency. In addition, the transcriptional promoters of these genes can be used to drive the expression of foreign genes inserted into the viral genome.

Lymantria dispar nuclear polyhedrosis virus (LdNPV) early transcripts were identified through northern analysis. RNAs isolated from 652Y cells, infected with clonal isolate 5-6 (CI 5-6), 2 and 7 hours postinfection were probed with LdNPV genomic fragments from a cosmid library. Fifteen viral transcripts were detected: three were chosen for further study. A cDNA library was constructed (in lambda gt11) from poly A+ RNA isolated from 652Y cells 7 hours after infection with CI 5-6, and probed with LdNPV genomic fragments containing the coding sequences for the three genes of interest. Several positive plaques for each gene were identified and used for further study.

The clone lambda LdIE-I contains a cDNA of 880 bp and is derived from a transcript of approximately 950 bases in length. This gene, designated IE-I, is initially expressed 4 hours postinfection (p.i.), and is synthesized throughout infection at near steady state levels. At least three other distinct viral transcripts were identified that contain IE-I sequences. The approximate genomic location of the IE-I gene is from 6.0 to 6.7 map units. In addition, IE-I contains sequences with limited homology to the *Autographa californica* NPV gene IE-N. The clone lambda LdIE-G1 contains a cDNA of 750 bp that is derived from a gene (termed IE-G1) that codes for a transcript of approximately 750 bases in length. IE-G1 is expressed primarily from 2 to 10 hours p.i. (the transcript is detectable after a 1 hour adsorption period), and maps to the genomic area between 9.3 and 13.7 map units. The clone lambda LdIE-G2 contains a cDNA of 1950 bp that is derived from a gene (termed IE-G2) that codes for a transcript of approximately 2050 bases in length that maps to the genomic region between 9.3 and 13.7 map units. IE-G2 is expressed primarily from 4 to 10 hours p.i., and is first detected 2 hours p.i. In addition, at least 4 other viral transcripts overlap the IE-G2 gene.