

TEMPORAL ANALYSIS AND SPATIAL MAPPING OF *LYMANTRIA DISPAR*
NUCLEAR POLYHEDROSIS VIRUS TRANSCRIPTS AND *IN-VITRO* TRANSLATION
PRODUCTS.

James M. Slavicek and Nancy Hayes-Plazolles
USDA Forest Service, Northeastern Forest Experiment Station
359 Main Road, Delaware, OH 43015

ABSTRACT

The *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) is being used as a biopesticide against the gypsy moth. We are attempting to enhance the potency of the LdNPV through recombinant DNA technology. As a prerequisite to genetic manipulation, we have characterized LdNPV gene expression in cell culture through the generation of transcription and translation maps. In addition, LdNPV polypeptides expressed in cell culture were identified.

To generate a genomic transcription map, northern blots containing RNAs isolated from 652Y cells 2, 7, 16, 24, 48, and 72 hours after infection with clonal isolate (CI) 5-6, were probed with labelled DNA fragments covering the LdNPV genome. Sixty-six viral transcripts were sized and mapped onto the genome. Fifteen viral transcripts were expressed early in infection, while most viral RNAs were initially detected from 16 to 24 hours post-infection (p.i.), and continued to be expressed until late in infection.

LdNPV CI 5-6 polypeptides, synthesized in 652Y cells, were identified after labelling with 35-S methionine and SDS-polyacrylamide gel electrophoresis. The expression of viral protein occurred in a sequential manner: 4 polypeptides were synthesized from 4 to 16 hours p.i., 24 proteins were initially detected from 12 to 20 hours p.i., and 5 polypeptides were initially detected at 24 hours p.i. These results are in good agreement with the study by J. McClintock et. al. (Virus Res., 5:307-322, 1986).

A translation map of LdNPV CI 5-6 was constructed. Six overlapping DNA fragments encompassing the CI 5-6 genome were used to hybrid select viral transcripts from RNA isolated from infected 652Y cells 7, 16, 24 and 48 hours p.i. The selected transcripts were translated in reticulocyte lysates in the presence of 35-S methionine, the protein products separated by SDS polyacrylamide gel electrophoresis, and the labelled proteins visualized by autoradiography. The approximate genomic location of 89 LdNPV translation products were mapped. No proteins were detected using RNA hybrid selected from cells 7 hours p.i. *In-vitro* translation products were generated using RNAs from cells 16, 24 and 48 hours p.i. Eighteen proteins were expressed from 16 to 48 hours p.i., 5 proteins from 16 to 24 hours p.i., and 32 proteins from 24 to 48 hours p.i. Five proteins were detected only with RNA isolated 16 hours p.i., 22 proteins were specific to RNA isolated 24 hours p.i., and 7 proteins were specific to RNA isolated 48 hours p.i. The genes coding for proteins expressed late in infection were found to be dispersed throughout the LdNPV genome.