Transcriptional analysis of *Choristoneura fumiferana* multiple nucleopolyhedrovirus (CiMNPV) genes and antisense transcripts by an oligonucleotide-based two-channel DNA microarray

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### INTRODUCTION

The spruce budworm, *Choristoneura fumiferana*, is historically the most destructive insect in northern and spruce forests in Eastern Canada. The use of CiMNPV as a specific biological control agent is promising in integrated pest management. The CiMNPV 129 593 bp long double-stranded DNA genome encodes 146 computational open reading frames (ORFs) including seven unique ORFs. The transcription of CiMNPV is temporally regulated into IE (immediate-early), E (early), L (late), and YL (very late) phases. The transcription pattern and the functions of the majority of the CiMNPV genes have not yet been described. In this work, we report the temporal transcription of select CiMNPV ORFs focusing on its 7 unique ORFs using oligonucleotide-based two-channel DNA microarray. To identify any antisense, presumably, non-protein-coding RNAs that may play important roles in viral infection, we analyzed antisense transcription of 23 select CiMNPV ORFs with microarray chips containing oligonucleotide probes for their complementary strands. We also analyzed the expression profiles of five host insect genes to explore the possibility of using them as internal controls. We used CiMNPV viral genomic DNA (vgDNA) as a reference standard to generate more reliable data. The microarray results provided some interesting insights into the temporal expression of these select viral genes and evidence of antisense transcription.

### RESULTS

#### 1. Viral genomic DNA as a standard for gene expression analysis

To overcome the inherent variability problem of the traditional microarray procedure, we tested a protocol using CiMNPV vgDNA as a reference standard for data normalization by comparing gene expression levels to the signals produced from co-hybridizing vgDNA (Fig. 1). To optimize the co-hybridization conditions, different amounts of Cy5 labelled vgDNA derived from Nick translation reactions were co-hybridized with a fixed amount of Cy5 labelled CDNA synthesized from total RNA.

#### 2. Expression analysis of 7 CiMNPV unique ORFs and 5 host insect genes

![Fig. 2. Genomic normalization of gene expression. (A) Expression profiles of two CiMNPV very late genes at 48 hours post infection (hpi) co-hybridized with increasing amounts of Cy5 vgDNA as a 0.147 pg/μl (A) The percentage of CiMNPV select genes which could be detected using varying amounts of Cy5 vgDNA as standards. The Cy3 cdNA was co-hybridized with increasing amounts at 48 hpi, where most of the CiMNPV genes would be expressed at various levels.

![Fig. 4. Profiles of antisense transcription detected by DNA microarray for 23 select CiMNPV ORFs. The experimental conditions were the same as described in Fig. 3. The asterisk indicates expression of the non-coding strand (nc) of the polyhedrin gene. The complements are shown in the order of increasing intensities at 24 hpi.

#### 3. Detection of CiMNPV antisense transcripts

![Fig. 3. (A) Transcription profiles of 7 CiMNPV unique ORFs. Total RNA was isolated from CIf03 cells at 0, 3, 6, 12, 24, and 48 h post infection. The Cy3 labelled cdNA derived from 20 ng total RNA was co-hybridized with 10 ngCy5 vgDNA to each array at 4°C. (B) Expression levels of 5 host cell genes, actin (a), GAP (g), ß-Actin (act), and EF1-γ (e), as measured by direct probe fluorescence.

#### 4. Mapping of the 5’ and 3’ ends of the polyhedrin antisense transcript

![Fig. 5. Long range RT-PCR analysis of the polyhedrin antisense transcript. The genome sequence flanking polyhedrin is presented (A) for the relative locations of primers, polyk signals, TATA box and an early promoter (CAGT). The putative transcript of ORF146 (solid arrow) and the respective cdNA templates (dashed arrows) are shown. The 5’ site (B) and the 3’ site (C) of the antisense transcript were mapped by RT-PCR using specific cdNA templates with several primer pairs as indicated. M: 1 kb ladder; C: cdNA; N: RT-negative control; V: vgDNA; P: primer control.

### SUMMARY

- Transcription of 7 CiMNPV unique ORFs was detected for the first time using DNA microarray analysis. Their temporal expression profiles suggest that they are functional and could be involved in both early and late phases of viral infection.
- The expression of four of the five host insect genes varied several fold throughout virus infection and therefore were unsuitable for normalization between arrays.
- Detection of antisense transcription of CiMNPV select genes emphasizes the added value of using single-stranded oligonucleotides instead of amplicons as probes.
- Based on the long-range RT-PCR analysis, the polyhedrin antisense transcript appeared to be a read-through product of ORF146.
- The viral genomic DNA standard offers advantages in overcoming the variability problem of the traditional normalization protocols.