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Transcription of Some Early and Late Genes of *Bombyx mori* Nuclear Polyhedrosis Virus in the Cells

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ABSTRACT

To understand expression of some early and late genes of *Bombyx mori* nuclear polyhedrosis virus (BmNPV) in the *B. mori*-derived BmN cell line, the transcripts were analyzed by polymerase chain reaction with synthetic primers. After infection, the transcript of early genes, which include p35, IE1 and helicase p143, was immediately detected in the infected cells. In addition, the transcript of late genes, which include p10 and polyhedrin, was also detected in just-infected cells. In conclusion, our results revealed that transcripts of early and late genes of BmNPV are immediately expressed from the cells after infection.

Key words : *Bombyx mori* nuclear polyhedrosis virus, Early gene, Late gene, Insect cells

INTRODUCTION

Baculoviruses are characterized by large, double-stranded DNA genomes and rod-shaped, enveloped virions. Baculoviruses originally centered on their natural ability to control insect pest populations, and they have recently been used as expression vectors for the production of proteins for medical research and biotechnology (Blissard & Rohrman, 1990).

Baculoviruses are divided into three subgroups. Subgroup A, the nuclear polyhedrosis viruses (NPVs), have many virions occluded within single intranuclear crystals called polyhedra. Among them, *Autographa californica* NPV (AcNPV) and BmNPV have successfully been investigated. The entire genome of AcNPV and BmNPV has recently been sequenced (Ayres *et al.*, 1994; Maeda, 1995).

Baculovirus gene expression is divided into two general phases, an early phase that precedes viral DNA replication and a late phase that occurs as or after viral DNA replication begins. In the infected insect cells, the expression of viral genes and DNA replication are believed to occur in an ordered cascade of events in which each successive phase is dependent on the previous phase (Blissard & Rohrman, 1990; Kool *et al.*, 1995).

In the BmNPV, BmNPV T3 strain have been sequenced and developed as expression vector (Maeda *et al.*, 1985; Maeda, 1995). In this study, we analysed expression of some early and late genes of BmNPV vB2, which was isolated from the silkworm *B, mori* in Korea, in the *B. mori*-derived BmN cells.

MATERIALS AND METHODS

1. Cell line and virus

B. mori cells (BmN-4) used in this study were maintained at 27°C in TC-100 medium (Sigma) supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (FBS, Gibco). Wild-type *Bombyx mori* nuclear polyhedrosis virus vB2 (Kang *et al.*, 1997) was propagated in BmN-4 cells. The titer of virus was determined by plaque assay in BmN-4 cells as described previously (O'Reilly *et al.*, 1992)

2. RNA extraction

BmN-4 cells were infected with wild-type BmNPV in a 35 mm diameter dish (2×10^6) at an MOI of 5

Table 1. *Primers used in this study*

Primer	Position	Nucleotide sequence
	+29~+45	5'-TACACCAGTGCTCCGA-3'
IE1	+734~+751	5'-ACCACATTGCTCACGTA-3'
	+1341~+1365	5'-GGCTATTATTTTGGCATACACTACA-3'
p143 helicase	+1817~+1841	5'-CACATTTTGGCGATGTGTTAAGTTC-3'
	+37~+52	5'-AACTGCAGACGGTTATTCGAG-3'
p35	+745~+761	5'-CAGCTCGATTCTGTAG-3'
	+3~+21	5'-TCCCCCGGGTCAAAGCCTAACGTTTTG-3'
p10	+263~+278	5'-TGGAACTGCGTTTACC-3'
	+34~+53	5'-CGTACTTACGTGTACGACAA-3'
polyhedrin	+556~+575	5'-TCGAACGAGTTGGTGTACTC-3'

PFU per cell. After incubation at 27°C, cells were harvested at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 12, 18, 24, 36 and 48 hr postinfection (p.i.). Cells infected with or without virus were washed twice with phosphate-buffered saline (PBS; 140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNA was isolated from the cells as described by Sambrook *et al.* (1989).

3. PCR

The first stranded DNAs from the total RNA were synthesized by reverse transcription reaction. The primers for the early and late genes of BmNPV used in PCR were listed in Table 1 (Maeda *et al.*, 1985; 1993; Kang *et al.*, 1997; Huybrechts *et al.*, 1992).

After a 35-cycle amplification (94°C for 1 min; 55°C for 1 min; 72°C for 1 min), PCR products were ethanol precipitated, centrifuged at 10,000g for 30 min, and rinsed with 70% ethanol. These DNAs were analyzed by agarose gel electrophoresis.

4. DNA sequencing

Nucleotide sequence of PCR product was determined by the dideoxy chain termination method (Sanger *et al.*, 1977).

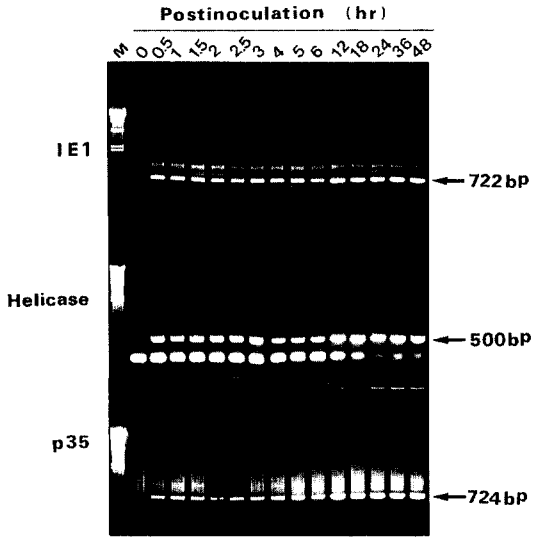

Fig. 1. *Expression of transcipts of early genes of BmNPV vB2 in the cells. The transcript of p35, IE1 and helicase p143 genes of BmNPV vB2 in the BmN cells was analysed by PCR. An expected size of PCR products is indicated on the right of each panel.*

RESULTS AND DISCUSSION

To understand expression of some early and late genes of BmNPV in the *B. mori*-derived BmN cell line, the transcripts were analyzed by PCR with synthetic primers listed in Table 1. After infection, the transcript of early genes, which include p35, IE1 and helicase p143, was immediately detected in the infected cells (Fig. 1). There were similar tendency in the expression level for p35, IE1 and helicase p143.

Furthermore, the transcript of late genes, which include p10 and polyhedrin, was analyzed in infected cells (Fig. 2). Interestingly, the transcript was also detected from 30 min p.i. It indicated that transcripts of early and late genes of BmNPV were immediately detected from just-infected cells. In case of PCR with polyhedrin gene primers, about 400 bp fragment was detected from mock-infected cells and infected cells

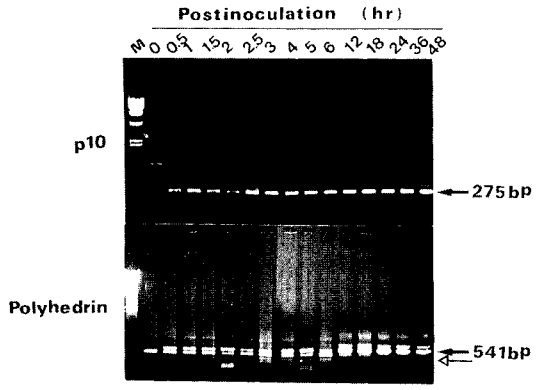

Fig. 2. *Expression of transcipts of late genes of BmNPV vB2 in the cells. The transcript of p10 and polyhedrin genes of BmNPV vB2 in the BmN cells was analysed by PCR. An expected size of PCR products is indicated on the right of each panel.*

by 12hr p.i. The sequence analysis revealed that the fragment have homology with *B. mori* vitellin degrade enzyme precursor and α-amylase genes (data not shown), suggesting that genomic DNA of host cells was degraded by virus replication.

In fact, baculovirus gene expression occurs in an ordered cascade of events, beginning with the expression of early genes. Subsequently, the expression of late and very late genes occurs either concurrently with or after the initiation of viral DNA replication (Blissard & Rohrman, 1990; Kool *et al.*, 1995).

In this study, we found that after infection, early and late genes of BmNPV were immediately expressed in the cells. These findings are consistent with those of AcNPV (Blissard & Rohrman, 1990; Kool *et al.*, 1995), and suggest that an increased understanding of some early and late genes of BmNPV vB2 should provide as information for the BmNPV vB2 replication in the cells.

적 요

누에 유래 BmN 세포주에서 누에 핵다각체병 바이러스 (BmNPV vB2)의 몇가지 초기 및 말기 발현 유전자의 발현을 구명하기 위하여, 감염 후 그 전사체들을 PCR로 분석하였다. p35, IE1과 helicase p143 등의 초기 발현 유전자는 감염 후 즉시 감염세포주에서 발현되었다. 또한 p10과 다각체 단백질 유전자 등의 말기 발현 유전자 역시 감염된 세포주로부터 초기 발현 유전자의 발현과 함께 그 발현이 확인되었다. 결론적으로 세포주에 감염 후 즉시 누에 핵다각체병 바이러스의 초기 발현 유전자 발현과 더불어 말기 발현 유전자 역시 발현됨을 확인되었다.

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