Molecular Cloning and Characterization of Very Late Expression Factor 1 Gene, vlf-1 from Bombyx mori Nuclear Polyhedrosis Virus K1

Hye Jin Park, Kwang Sik Lee, Eun Sook Cho, Eun Young Yun, Seok Woo Kang, Keun Young Kim, Hung Dae Sohn and Byung Rae Jin*

College of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea.
1Dept. of Sericulture and Entomology, The National Institute of Agricultural Science and Technology, RDA, Suwon 441-744, Korea.

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We cloned and characterized a very late expression factor 1 gene, vlf-1, which regulates the level of very late gene transcripts, from Bombyx mori nuclear polyhedrosis virus (BmNPV) K1 strain. The 1,140 bp vlf-1 has an open reading frame of 379 amino acid and a MW of 44 kDa. The vlf-1 nucleotide sequence of BmNPV-K1 showed high homology with Autographa californica nuclear polyhedrosis virus and BmNPV T3 strain so far known, and its deduced amino acid residues were identical to those of BmNPV T3. The location of vlf-1 in the BmNPV-K1 genome was confirmed by Southern blot analysis and its expression patterns at the transcriptional level were confirmed by Northern hybridization analysis.

Key words: Baculovirus, Bombyx mori nuclear polyhedrosis virus, Very late expression factor 1 gene (vlf-1)

Introduction

Baculoviruses possess a large circular DNA genome which replicates in the nuclei of infected cells and is transcribed in three temporally distinct phases: early, late, and very late. The promoters of the genes encoding the polyhedrin and p10 proteins of baculovirus are most frequently employed in baculovirus expression vector systems to express heterologous gene (King and Possee, 1992; O'Reilly et al., 1992). Both promoters are strongly activated during the very late stage of infection, which are activated at between 18 and 24 hrs postinfection (p.i.).

Late gene transcription is mediated by a novel, α-amanitin-resistant RNA polymerase activity which is induced during virus infection (Glocker et al., 1993; Grula et al., 1981; Huh and Weaver, 1990a, 1990b) and is probably encoded, at least in part, by the viral genome (Passarelli et al., 1994). Very late gene expression, which is required for occluded virus formation, is also mediated by an α-amanitin-resistant RNA polymerase but additionally requires the function of a novel gene, very late expression factor 1 gene (vlf-1), which is predicted to encode a polypeptide with sequence motifs characteristic of a family of integrase/resolvases (McLachlin and Miller, 1994). The promoters of most late and very late genes have novel properties, including an absolute dependence on a TAAG sequence located at the initiation point of transcription (Morris and Miller, 1994; Ooi et al., 1989).

The vlf-1 previously identified by analysis of a temperature-sensitive mutant of Autographa californica nuclear polyhedrosis virus (AcNPV) (McLachlin and Miller, 1994) in the transient-expression assay and found that vlf-1 specially transactivated the very late promoters and VLF-1 is the primary regulator of very late gene expression (Todd et al., 1996). Thus, the vlf-1 is required for strong expression of the polyhedrin gene and is expressed primarily as a late gene. By altering the level and/or timing of vlf-1 expression, the timing of polyhedrin gene (polh) expression, which normally occurs very late in infection, could be advanced or delayed (Yang and Miller, 1998a). Early overexpression of vlf-1 increased the level of expression from the polh promoter. Because expression of polh responds to expression of vlf-1, VLF-1 can provide a means of regulating baculovirus expression vector systems employing the polh promoter to drive foreign gene expression (Yang and Miller, 1998b).

In this study, we have cloned and characterized vlf-1 from Bombyx mori nuclear polyhedrosis virus K1 strain

*To whom correspondence should be addressed.
College of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea. Tel: +82-51-200-7594; Fax: +82-51-200-7594; E-mail: brjin@mail.donga.ac.kr
Materials and Methods

Cells and virus
The Spodoptera frugiperda IPLB Sf21-AE (Vaughn et al., 1977) clone 9 (Sf9) and Bombyx mori 5 (Bm5) (Grace, 1962) cells were grown at 27°C in TC-100 medium (GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL) (O’Reilly et al., 1992). Wild-type AcNPV (Lee and Miller, 1978) was propagated in Sf9 cells. Wild-type BmNPV-K1 (Kang et al., 1997) and BmNPV T3 (Gomi et al., 1999) were propagated and titrated in Bm5 cells. The titer was expressed as plaque forming units (PFU) per ml (O’Reilly et al., 1992).

Viral genome isolation
Polyhedra and viral DNA were obtained from Bm5 cells by standard methods (O’Reilly et al., 1992). Polyhedra were purified by centrifugation through discontinuous 40 to 65% sucrose gradients. Viral DNA was isolated from purified polyhedra by proteinase K digestion followed by phenol extraction (O’Reilly et al., 1992).

Polymerase chain reaction (PCR)
Viral DNAs were used as templates. The vlf-I was amplified from viral DNAs using the primer 5’-GATAGATTGACACCAGATTCTCC-3’ and 5’-CCCTTACTCTATATCGTGGCG-3’, annealing to the 5’ promoter region and 3’ untranslational region respectively (McLachlin and Miller, 1994). After 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 10,000 × g for 15 min, and rinsed with 70% ethanol. These DNAs were analyzed by 1% agarose gel electrophoresis. The PCR products for sequencing were cloned into pGEM-T vector (Promega).

DNA sequencing
By utilizing double-stranded DNA templates synthesized by PCR, both strands were sequenced across the entire region by the dideoxynucleotide chain termination method (Sanger et al., 1977).

Multiple sequence alignment
Protein sequence homology searches were performed by using the predicted amino acid sequence of VLF-1 (accession number S36692; AcMNPV hypothetical protein ORF 1137) and the basic local alignment search tool (BLAST) (Altschul et al., 1990) to search the National Center for Biotechnology Information nonredundant peptide sequence database. Sequence alignments were conducted by using the Pileup multiple sequence alignment program of the Genetics Computer group (Madison) sequence analysis software package. The following list includes the accession numbers for the sequences used in the multiple sequence alignments; the sequences were derived from either the GenBank or Swiss-Prot database: VLF-1 AcMNPV (S36692); vlf-I AcMNPV (L22858); and vlf-I BmNPV T3 (L33180).

Southern blot analysis
Viral DNAs digested with EcoRV and SalI were electrophoresed through 1.0% agarose gel as described previously (O’Reilly et al., 1992). The DNA of the gel was transferred onto a nylon blotting membrane (Schleicher & Schuell) and hybridized at 42°C. The probe used to detect DNA fragment containing vlf-I was a 1140 bp BmNPV-K1 vlf-I amplified by PCR in this study.

RNA isolation
Total cellular RNA was isolated from mock-infected or wild-type BmNPV-infected Bm5 cells. A total of 1 × 10⁶ cells per 35-mm-diameter dish was infected at a multiplicity of infection of 5 PFU per cell. Cells were collected at 4, 8, 12, 18, 24, and 48 hrs p.i. Total cellular RNA was isolated by use of a guanidinium isothiocyanate procedure.

![Fig. 1. PCR of vlf-I from the genome of baculoviruses. The PCR primers for identification of BmNPV-K1 vlf-I were based on the previously identified vlf-I within 45.2 to 49.6 map units of AcMNPV (A), as described in Materials and Methods. The amplified PCR products were analyzed by 1% agarose gel electrophoresis (B). AcNPV (lane 1), BmNPV T3 (lane 2), Bm NPV-K1 (lane 3), and DNA size markers (M) are indicated.](image-url)
**Molecular cloning and characterization of vlf-I**

![Fig. 2. Nucleotide (A) and deduced amino acid (B) sequences of BmNPV-K1 *vlf-I*. The sequences of BmNPV-K1 were compared with those of AcNPV and Bm NPV T3. The differences between BmNPV-K1 and Bm NPV T3 sequ-ences are indicated in boldface at nucleotides 108, 303, and 351. The nucleotide sequence data of BmNPV-K1 *vlf-I* have been deposited with the EMBL/GenBank libraries under the accession number AF 191747.](image)

(Chirgwin et al., 1979).

**Dot blot analysis**

Total cellular RNA (1 µg per well) from infected cells was denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell) and hybridized at 42°C in the presence of 50% formamide. The probe used to detect *vlf-I* transcripts was a 1140 bp BmNPV-K1 *vlf-I* amplified by PCR in this study.

**Nucleotide sequence accession number**

The sequence data obtained from this study have been deposited with the EMBL/GenBank libraries under the
Results and Discussion

To identify vlf-1 in BmNPV-K1, we have designed the PCR primer set based on the sequences of the conserved region of vlf-1 of AcNPV and BmNPV T3 so far known (Fig. 1A). The amplified PCR products, as expected, were observed in three baculoviruses (Fig. 1B). As shown in Fig. 1, the molecular size of the products in three baculoviruses was identical to that expected. The PCR products for sequencing were cloned (data not shown).

The nucleotide sequence of PCR products was analyzed and its amino acid was deduced. As the result of the complete nucleotide sequence (GenBank accession number: AF191747) in Fig. 2, the 1,140 bp vlf-1 has an open reading frame of 379 amino acid and a predicted MW of 44 kDa. The nucleotide and deduced amino acid sequences were compared with those of AcNPV and BmNPV T3. The sequences of BmNPV-K1 showed high homology with AcNPV and BmNPV T3 strain so far known (McLachlin and Miller, 1994; Gomi et al., 1999). The vlf-1 of BmNPV-K1 was different from nucleotide sequences at positions, 108, 303 and 351 in BmNPV T3. However, deduced amino acid sequences of vlf-1 of BmNPV-K1 were identical to those of BmNPV T3.

The localization of vlf-1 in the BmNPV-K1 genome was confirmed by using Southern blot analysis. BmNPV-K1 genome was digested with EcoRV and SalI, and proved with amplified vlf-1 (Fig. 3). The vlf-1 in BmNPV-K1 genome was localized on the 2.8 kb EcoRV fragment and 13.8 kb SalI fragment.

To verify whether the vlf-1 transcripts were correlated with virus replication, we examined dot hybridization analysis with vlf-1 probe (Fig. 4). As shown in Fig. 4, vlf-1 transcripts were being dramatically transcribed in the wild-type BmNPV-K1-infected cells at 18 hr p.i. Thus, this result was consistent with the previous result that revealed a correlation between the timing of vlf-1 expression and the timing and/or level of polyhedrin and p10 synthesis in the wild-type AcNPV-infected cells (Yang and Miller, 1998b). Actually, the fact that transcription from the polh and p10 promoters is strongly activated at 18 and 24 hrs p.i was previously reported (King and Possee, 1992; O’Reilly et al., 1992). In addition, the product of vlf-1 is known to be involved in the regulation of polyhedrin synthesis at the transcriptional level as a limiting factor in very late gene expression (Yang and Miller, 1998b).

Knowledge of the vlf-1 in this study will provide an information for establishing BmNPV-K1 strain. The vlf-1

![Southern blot analysis of BmNPV-K1 genome. Viral DNAs digested with EcoRV (lane 1) and SalI (lane 2) were electrophoresed through a 1.0% agarose gel (A) and hybridized at 42°C with a labeled probe (B). The probe used to detect DNA fragment containing vlf-1 was a 1140 bp BmNPV-K1 vlf-1 amplified by PCR in this study. The DNA size markers (M) are indicated in kilobases.](image)

![Dot blot analysis of vlf-1 transcripts from BmNPV-K1-infected cells. Total cellular RNA was collected from Bm5 cells at various times p.i. as indicated at the top of each well. The probe used to detect vlf-1 transcripts was a 1140 bp BmNPV-K1 vlf-1 amplified by PCR in this study.](image)

BmNPV-K1 will now provide a means of developing transformed B. mori cell line expressing vlf-1.

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References


