The pl10 gene of natural isolates of Bombyx mori nuclear polyhedrosis virus encodes a truncated protein with an \( M_r \) of 7700

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Sequence analysis of the pl10 genes of three Bombyx mori nuclear polyhedrosis virus (BmNPV) isolates collected in Taiwan (Ta) and Japan (T3 and D1) showed that all possessed a deletion of an adenine residue, 210 bp downstream from the first base of the initiation codon when compared to the pl10 gene of Autographa californica (multinucleocapsid) NPV (AcMNPV). This deletion caused a downstream termination codon to come in-frame with the coding sequence of pl10, so that the pl10 gene of BmNPV encoded a protein of 70 amino acid residues with an \( M_r \) of 7700. This is considerably shorter than the 10000 \( M_r \) protein encoded by the closely related AcMNPV.

Baculoviruses produce two morphologically distinct forms of progeny virus. Non-occluded viruses are released from infected cells during the early stage of infection, whereas progeny viruses are embedded in occlusion bodies during the late stage of infection (Kelly, 1982). Occlusion bodies are compared primarily of a protein called polyhedrin, the mRNA of which is transcribed under the influence of a strong promoter during the very late stage of infection (Vlak et al., 1981; Rohel et al., 1983). A second gene (p10), with a promoter that is probably equally strong, is activated simultaneously (Vlak et al., 1981; Rohel et al., 1983; Roelvink et al., 1992). The p10 gene of Autographa californica (multinucleocapsid) nuclear polyhedrosis virus (AcMNPV) expresses a 10K protein of 94 amino acid residues (Kuzio et al., 1984). Insertion of the Escherichia coli lacZ gene in frame with the coding sequence of the p10 gene of AcMNPV does not affect virus production (Vlak et al., 1988). Furthermore, a p10-\( \beta \)-galactosidase fusion protein is synthesized in amounts that can be visualized in a Coomassie Brilliant Blue-stained gel (Vlak et al., 1988). These results suggest that the p10 gene is not essential for virus replication, but the p10 promoter is effective for producing large quantities of heterologous proteins. Indeed, an expression vector utilizing the p10 promoter has been used successfully to express cauliflower mosaic virus gene I (Vlak et al., 1990).

Using antiserum against the p10 protein in immunogold labelling experiments, van der Wilk et al. (1987) observed gold labelling of fibrillar structures in the nucleus and cytoplasm of infected cells. Interestingly, such fibrillar structures were replaced with granular structures when the lacZ gene was inserted in frame within the p10 coding sequence (Vlak et al., 1988; Williams et al., 1989). Although the granular structures may have resulted from overproduction of the p10-\( \beta \)-galactosidase fusion protein, it is likely that the fibrillar structures are principally composed of the p10 protein. Recent studies by van Oers et al. (1993) further suggest that the C terminus of the p10 protein is required for the formation of fibrillar structures. Phase-contrast microscopic examinations of cells infected with AcMNPV with a mutation in the p10 gene suggest that the p10 protein may also be involved in the lysis of infected cells (Williams et al., 1989) or, more precisely, the disintegration of the nucleus (van Oers et al., 1993).

Bombyx mori nuclear polyhedrosis virus (BmNPV) is a member of the Eubaculovirinae subfamily of the Baculoviridae (Francki et al., 1991). NPVs are characterized by polyhedron-shaped occlusion bodies containing one or many enveloped virions. The nucleocapsids are enveloped singly (SNPV) or multiply (MNPV). Although BmNPV is classified as an SNPV (Francki et al., 1991), an MNPV form has been observed in both infected silkworm larvae and infected cell lines (Kawarabata &
AcMNPV
BmNPV Ta  TCGAGCAAGAATAAATAAACGCAAACGCGTTGAGCTTGTTGCTAATTTTACAGAGATTCAGAAATACG -161
BmNPV T3  G
BmNPV D1  G

AcMNPV
BmNPV Ta  CTCGCTAATAAGATAATATATTAGATATATATATGTAATTATAATGTTATACGTCAATATTTATTAATATT -81
BmNPV T3  C  C  T  T  T  CT
BmNPV D1  C  C  T  T  T  CT

AcMNPV
BmNPV Ta  A
BmNPV T3  A
BmNPV D1  A

ACAGCTAAATAAGATAATATATTAGATATATATGTAATTATAATGTTATACGTCAATATTTATTAATATT -1

AcMNPV
BmNPV Ta  GAGA
BmNPV T3  GAG
BmNPV D1  GAG

GTAAACGGGCTGAAATGCACTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +160

ACAGCTAAATAAGATAATATATTAGATATATATGTAATTATAATGTTATACGTCAATATTTATTAATATT -1

AcMNPV
BmNPV Ta  GAGA
BmNPV T3  GAG
BmNPV D1  GAG

AAATTCATGCTGACAGTCTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +240

AcMNPV
BmNPV Ta  CTTCTCTTTGAGTCAAAACGCTAAGTTTTGACAGAATTTAGACGCATTGGGAAAAAGGTTGACAGTGTCAAACTA +80
BmNPV T3  A
BmNPV D1  A

GTTAAACGGGCTGAAATGCACTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +320

AcMNPV
BmNPV Ta  GAGA
BmNPV T3  GAG
BmNPV D1  GAG

AAATTCATGCTGACAGTCTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +500

AcMNPV
BmNPV Ta  CTTCTCTTTGAGTCAAAACGCTAAGTTTTGACAGAATTTAGACGCATTGGGAAAAAGGTTGACAGTGTCAAACTA +80
BmNPV T3  A
BmNPV D1  A

GTTAAACGGGCTGAAATGCACTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +320

AcMNPV
BmNPV Ta  GAGA
BmNPV T3  GAG
BmNPV D1  GAG

AAATTCATGCTGACAGTCTGCTCCCTGGGCTCAATTGGACACGGTATCTTAACACTTAATTCAG +500


Comparison of the p10 gene sequences of three different BmNPV isolates (Ta, isolated in Taiwan and D1 and T3, isolated in Japan) with that of AcMNPV indicated that an adenine was deleted 210 bp downstream of the first base of the initiation codon in each of the BmNPV p10 genes (Fig. 1a). The resulting frameshift brought a termination codon into the same reading frame as the coding sequence of the BmNPV p10 gene, thereby creating a C-terminally truncated p10 protein of 70 amino acid residues (Fig. 1b). Despite the relatively high homology among the four p10 genes throughout the coding sequence, the deletion was conserved in all three of the BmNPV p10 genes.

SDS-PAGE analysis of total cell extracts of BmN cells infected with the BmNPV Ta isolate collected at 48 h post-infection (p.i.) revealed a protein band with an
apparent $M_r$ of approximately 7700 (Fig. 2). This protein was not observed in extracts prepared from BmN cells infected with BmNPV whose p10 gene had been replaced (Fig. 2). Direct sequencing of a PCR-amplified DNA fragment of the p10 region of the BmNPV Ta also showed the same deletion at the same location, suggesting that the mutation was not a cloning artefact. These results confirmed that the p10 gene of BmNPV encodes a protein with an $M_r$ of 7700. Fibrillar structures were not observed in BmN cells infected with BmNPV T3 and D1 isolates (Inoue & Mitsuhashi, 1984; S. Maeda, unpublished results). These results are in agreement with the observation that the C terminus of the p10 of AcMNPV is required for the formation of fibrillar structures (van Oers et al., 1993).

Comparison of the three BmNPV isolates indicated that the Ta and T3 isolates were identical in the p10 gene coding sequences, but they differed from the D1 isolate by one base substitution within the last codon (Fig. 1a). This caused a single amino acid change in D1 (Fig. 1b). In addition to truncation of 24 C-terminal amino acid residues, the p10 gene products of BmNPV also contained eight amino acid substitutions compared to that of AcMNPV (Fig. 1b). In the 5' non-coding region, up to 230 bp upstream of the first base of the initiation codon, the nucleotide sequences of the p10 genes of the Ta and D1 isolates were identical and differed from that of the T3 by only one nucleotide (position −97). The consensus very late gene promoter sequence, TAAG, was conserved in all of the p10 genes. In the 3' non-coding region, sequences up to 222 bp downstream from the last base of the termination codon of the BmNPV p10 gene in the Ta and D1 isolates were identical, but differed from T3 at two sites, a two-base substitution at +229 and a two-base deletion at +301 (Fig. 1a). Of the five single-base differences in the 3' non-coding region of AcMNPV and the three BmNPV isolates, two deserve special consideration. The first generated an EcoRI site in BmNPV, which is absent in AcMNPV (Fig. 1a). The second created a putative polyadenylation signal 90 bp downstream of the termination codon of the p10 gene of BmNPV. The spacing between the termination codons and the putative polyadenylation signals located upstream of the p10 genes of BmNPV and AcMNPV was similar (90 and 88 bp, respectively).

In summary, sequence analysis of the p10 genes of the three different BmNPV isolates revealed only minor differences. But, despite the overall nucleotide sequence similarity between the AcMNPV and BmNPV p10 genes, a single-base deletion within the coding region of the p10 genes of BmNPV caused a dramatic change in the size of the p10 gene product of BmNPV. Absence of the C-terminal 24 amino acids of the p10 proteins of BmNPV was correlated with the absence of fibrillar structures in BmNPV-infected BmN cells. The significance of the fibrillar structures remains obscure, but they appear to be unrelated to cell lysis since the extent of polyhedra release in wild-type BmNPV Ta-infected cells is indistinguishable from that observed in wild-type AcMNPV-infected cells (Y.-F. Lu, R. Hou & N.-T. Hu, unpublished results). It has also been observed that removal of the C-terminal 15 amino acid residues of the p10 of AcMNPV blocked the formation of the fibrillar structures but not the release of polyhedra (van Oers et al., 1993). It would be interesting to see whether reintroduction of the A residue at position 210 would result in fibrillar structure formation.

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References


Short communication


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