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Bombyx mori nucleopolyhedrovirus *orf8* encodes a nucleic acid binding protein that colocalizes with IE1 during infection

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Summary. This report describes the characterization of the *Bombyx mori* nucleopolyhedrovirus (BmNPV) *orf8* gene. Immunoblot analyses demonstrated that *orf8* was expressed as an early gene. The ORF8 protein accumulated in the nucleus, and was maintained at relatively constant levels from 4 to 24 h postinfection. Immunoblot analysis failed to detect ORF8 protein associated with budded virus and occlusion derived virus. In addition, immunohistochemical analysis by confocal microscopy showed that ORF8 protein colocalized with IE1 to specific nuclear foci throughout infection. To further examine the function of ORF8, a reporter gene was inserted into the *orf8* reading frame. One *orf8* disruption mutant (BmD8), which expressed the N-terminal half of ORF8, was isolated. However, it was not possible to isolate a null mutant, suggesting that *orf8* may have an important role during viral infection. Single-step growth curves showed that BV production was reduced in BmD8 infected cells. Biochemical analyses indicated that ORF8 bound to nucleic acids. Together, these results suggest that BmNPV ORF8 may be involved in viral DNA replication and/or transcription.

Introduction

The family *Baculoviridae* is a large family of viruses that infect invertebrates, particularly insects of the order Lepidoptera. Baculoviruses contain circular double-stranded DNA genomes of 80 to 180 kb. They have been divided into two genera on the basis of their occlusion body morphology, the nucleopolyhedroviruses (NPVs), which have large occlusion bodies containing numerous virions, and the granuloviruses (GVs), which have single virions occluded within small granular occlusion bodies [31, 32]. The lepidopteran NPVs can be further subdivided into groups I and II based on phylogenetic studies [4, 8]. Recently, the complete

sequences of a number of baculovirus genomes have been reported. These include four group I NPVs, *Autographa californica* NPV (AcNPV), *Bombyx mori* NPV (BmNPV), *Orgyia pseudotsugata* NPV (OpNPV), and *Epiphyas postvittana* NPV (EppoNPV) [1, 2, 5, 10]. Comparison of these baculovirus genomes with the genomes of group II NPVs and GVs revealed that 17 genes are unique to group I NPVs [9]. The functions of some of these unique genes are known: *gp64* and *ptp1* encode structural proteins, *iap1* encodes an inhibitor of apoptosis, and *ie2* and *lef7* are involved in regulation of viral gene expression. Pearson et al. suggested that acquisition of *gp64* gene promoted the diversification of these viruses [28]. In addition, Herniou et al. suggested that acquisition of these unique genes may contribute to baculovirus speciation by causing alterations in host range [8].

The *orf8* gene of BmNPV is one of the 17 genes that are unique to group I NPVs. The homologues of BmNPV *orf8* are *orf16* of AcNPV (Ac16), *orf15* of OpNPV (Op15) and *orf13* of EppoNPV (Eppo13). The amino acid sequence of BmNPV *orf8* was 96% identical to that of Ac16 and showed 32 and 29% identity to that of Op15 and Eppo13, respectively. Op15 is 55% identical to Eppo13, and they are both smaller than that of ORF8 or Ac16 by 20 or more amino acid residues. The AcNPV homologue is also called *da26* or *bv/odv-e26* [3, 26]. The *da26* gene was shown to stimulate late gene expression in cooperation with *da41* in AcNPV [7]. O'Reilly et al. showed that *da26* is expressed as an early gene and that the encoded gene product is not essential for infectivity [26]. In addition, Beniya et al. reported that *da26* encodes an envelope protein common to budded virus and occlusion derived virus, and renamed the protein BV/ODV-E26 [3].

In this study, we examined the expression and localization of *orf8* in BmNPV infected BmN cells and found that ORF8 protein was not associated with the virion of BmNPV. Instead, we found that ORF8 colocalized with IE1 to specific nuclear sites throughout infection.

Materials and methods

Virus and cell lines

The BmN-4 (BmN) cell line was maintained in TC-100 with 10% fetal bovine serum as described previously [17]. The BmNPV T3 isolate [19] and recombinant virus were propagated on BmN cells as described [18]. Viral DNA used for generation of recombinant virus was purified from BmNPV T3 following a standard protocol [16]. Budded virus (BV) or occlusion derived virus (ODV) was purified from harvested supernatants of infected cells or hemolymph of infected larvae respectively as described by O'Reilly et al. [25]. For analysis of structural proteins, BVs or ODVs were purified by centrifuging through a 25 to 60% sucrose gradient [25] and verified by western analysis using antiserums of anti-BV or anti-ODV of BmNPV (gift from M. Nagata, The University of Tokyo).

Antibody production and immunodetection

The BmNPV *orf8* gene was amplified by PCR using an upstream primer (5′-CAGTCGACC AATTCTGTTCACACGC-3′) incorporating a *Sal*I site (underlined) and a downstream primer (5′-CAGCGGCCGCTTAATAGGCGTTAATATC-3′) with a *Not*I site (underlined). The amplified fragment was digested with *Sal*I and *Not*I and fused in frame with a hexa-histidine

tag (His-tag) in pET-28c(+) vector (Novagen). After expression of His-tagged ORF8 in *Escherichia coli*, recombinant ORF8 (rORF8) protein was purified using His Bind Resin (Novagen). Purified rORF8 was then used to raise polyclonal antibodies in rats. Subcellular fractionation and immunoblotting were performed as described [13, 33]. Immunohistochemistry and confocal microscopy were performed as described by Kang et al. [14] and Okano et al. [27]. For immunohistochemistry, anti-ORF8 antiserum (1:200 dilution), and Cy-5-conjugated goat anti-rat IgG (1:200 dilution; Jackson Immuno Research) or fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG (1:500 dilution; Cappel) were used. Anti-BV/ODV-E26 antiserum (1:1000 dilution, a generous gift from S. C. Braunagel, Texas A&M University), and FITC-conjugated goat anti-rabbit IgG (1:500 dilution; Cappel) were also used. IE1 was detected using anti-IE1 antiserum (1:200 dilution, a generous gift from L. Guarino, Texas A&M University), and Cy-5-conjugated goat anti-rabbit IgG (1:200 dilution; Amersham Life Sciences).

Construction of orf8 deletion mutant

Mutants were constructed by inserting a *β-galactosidase* gene cassette containing a *Drosophila melanogaster* heat shock promoter into the *orf8* open reading frame as described by Gomi et al. [6]. To construct a deletion of the entire coding region, a 2.3 kb *Cla* I-*Xba* I fragment containing *orf8* was purified from the genomic clone PstG [20] and inserted into pBluescript II SK- (Toyobo). Then, this plasmid was digested with *Eco*R I and *Nhe* I (Fig. 4A), and the resulting fragment was ligated with the *β-galactosidase* gene cassette. The resultant plasmid was co-transfected with wt BmNPV DNA into BmN cells using lipofectin (GibcoBRL). Recombinant BmNPV was isolated by identification of plaques expressing β-galactosidase as described [6, 18]. For construction of a C-terminal deletion mutant, PstG was digested with *Bbs* I and *Nhe* I (Fig. 4A) and ligated with the *β-galactosidase* gene cassette. Recombinant viruses were analyzed by PCR using *orf8* specific primers (see above), Southern hybridization, and western analysis.

Assay for BV production

For the virus growth curves, BmN cells were infected with either wt BmNPV or BmD8 at an MOI of 10. After 1 h of incubation, virus-containing medium was removed and fresh medium was added after washing monolayers twice with phosphate buffered saline (0 h postinfection). A small amount of culture medium was harvested at the designated times. BV production was determined by plaque assay.

Dot blot analysis

BmN cells were infected with either wt BmNPV or BmD8 at an MOI of 10. At designated times p.i., cells were harvested and subjected to dot blot analysis as described by Iwanaga et al. [12]. Probe DNA was prepared by PCR using two primers (5′-GGAATTCACGCAAATTAATTTTA ACGCGTCGTAC-3′, 5′-CGTCGACCCCTCGTCGATAATAAAAGA-3′) complementary to the *ie1* gene. Labeling of the probe with fluorescein-11-dUTP and hybridization were performed using ECL Direct System (Amersham Pharmacia Biotech). The analyses described above were repeated three times to confirm the reproducibility. The signals were analyzed using LAS-3000 (FUJIFILM) and quantified by Image Gauge software (FUJIFILM).

Column chromatography

Column chromatography using single strand (ss) DNA-cellulose, double strand (ds) DNA-cellulose or polyU-agarose (Sigma Aldrich) was performed as described previously [33].

Results

Western blot analysis of BmNPV ORF8

To address whether and when BmNPV *orf8* gene is expressed, we performed western blot analysis using nuclear and cytosolic fractions of infected cells and a polyclonal antiserum raised against recombinant ORF8 protein expressed in *E. coli*. An immunoreactive band with an apparent migration of 26 kDa was detected at 4 h p.i. in the nuclear fractions (Fig. 1). The migration of this protein was consistent with the computer-predicted molecular mass of 26,199 Da,

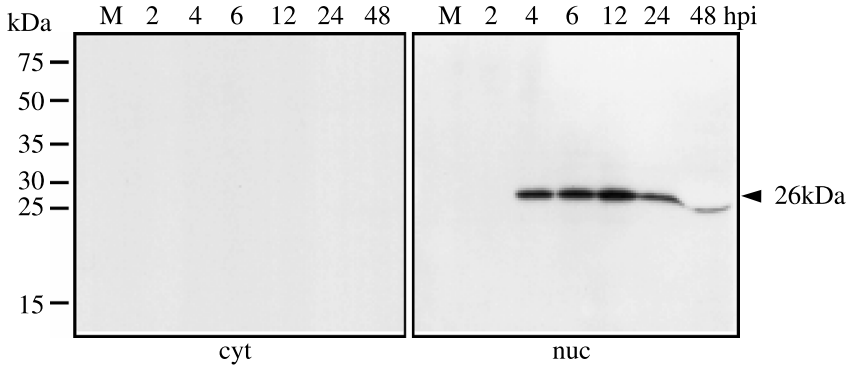


Fig. 1. Time course of ORF8 production by western blot analyses. Cytoplasmic (*cyt*) and nuclear (*nuc*) subcellular fractions of BmN cells harvested at the indicated times were subjected to western analysis. *M* indicates mock infection. The estimated size of immunoreactive bands is indicated on the right with an arrowhead. Size markers are indicated on the left side of the panels

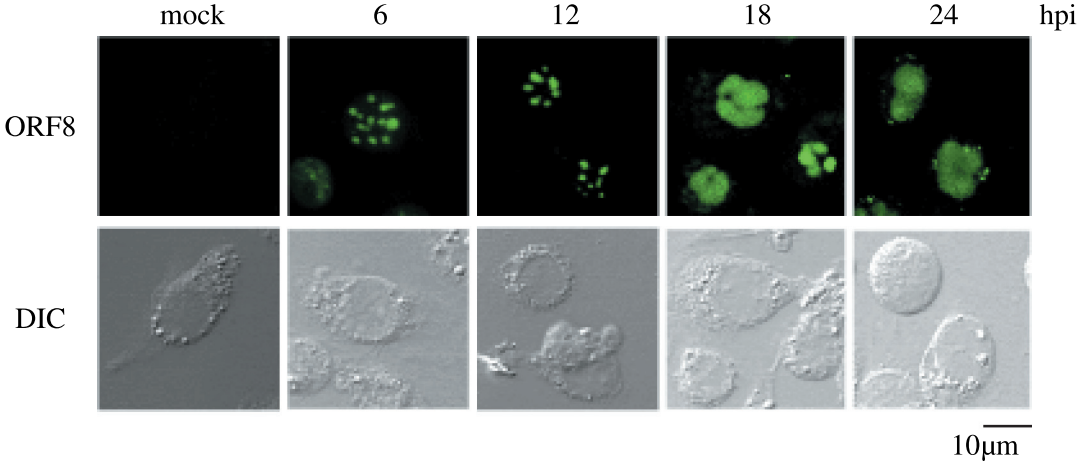


Fig. 2. Distribution of ORF8 in BmNPV infected BmN cells. Top panels show ORF8 immunofluorescence images and the lower ones show differential interface contrast images of the same fields as the top ones. BmN cells were mock infected or infected with BmNPV for 6, 12, 18 and 24 h, and subjected to immunohistochemistry. The scale bar is 10 μm

the levels of ORF8 peaked at 12 h p.i. and then declined at 24 and 48 h p.i.. No ORF8 was in cytosolic fractions at any point in the time course. Together, these results suggest that BmNPV *orf8* is expressed as an early gene and that it encodes a nuclear protein.

Nuclear localization of ORF8

Since subcellular fractionation analysis indicated that ORF8 is a nuclear protein, we further analyzed the localization of ORF8 within the nucleus by confocal microscopy. ORF8 accumulated within distinct foci early during infection (Fig. 2). These foci increased in size as infection progressed, eventually occupying most of the nucleus in the late phase. This localization pattern was very similar to that of BmNPV IE1, as previously reported [27]. Therefore, we performed a double labeling experiment using ORF8 and IE1 antisera to determine whether ORF8 colocalized with IE1 (Fig. 3). Colocalization of ORF8 and IE1 was evident even at 4 h p.i., when the foci were very small, and colocalization was observed through 20 h p.i.

Construction of orf8 deletion mutant

To further analyze the role of the BmNPV *orf8* gene during viral replication, we attempted to construct a viral mutant with the *LacZ* gene under the control of

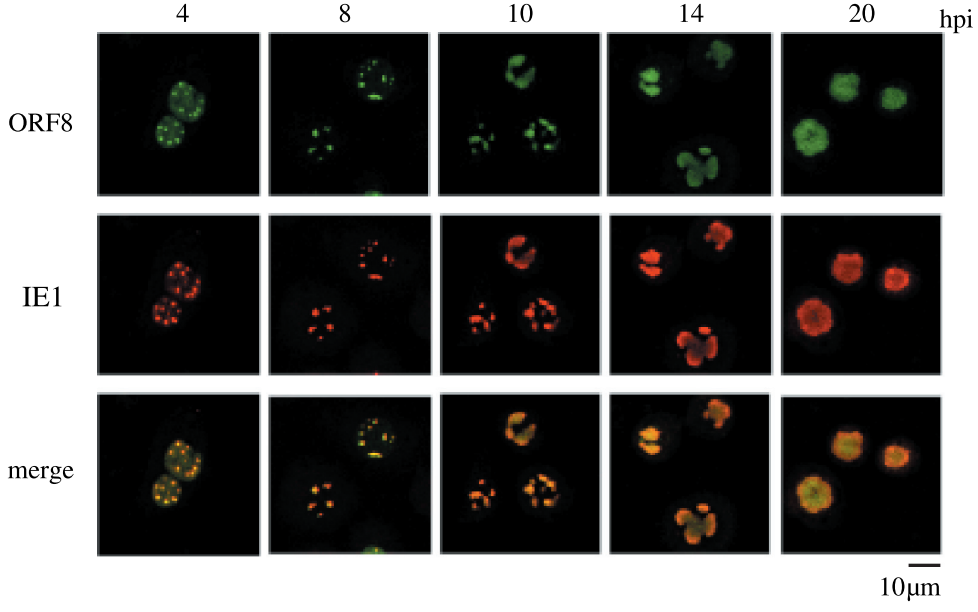


Fig. 3. Double staining of BmNPV infected BmN cells with ORF8 and IE1. Top and middle panels show ORF8 and IE1 immunofluorescence images, respectively. These were merged in bottom panels. BmN cells were infected with BmNPV for 4, 8, 10, 14 and 20 h, and subjected to immunohistochemistry. The scale bar indicates 10 μm

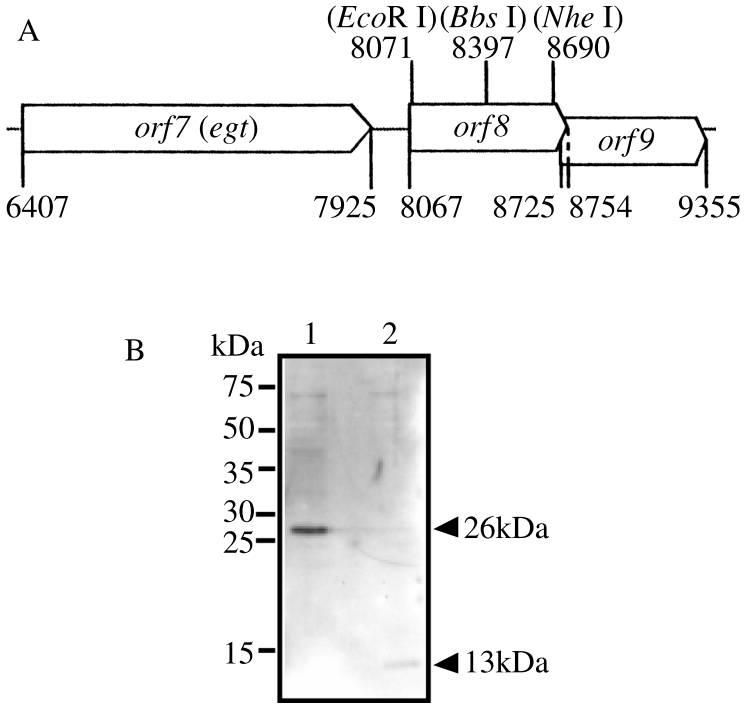


Fig. 4. Disruption of *orf8* gene. **A** Schematic representation of *orf8* in BmNPV genome. Positions and orientations of *orf7* (*egt*), *orf8*, and *orf9* are indicated by open arrows. The positions of restriction enzyme sites used for deletion of *orf8* gene are shown on the top. Numbers indicate the locations in the BmNPV genome. **B** Detection of ORF8 protein in BmD8 infected cells. Extracts of wt BmNPV (*1*) or BmD8 (*2*) infected cells were prepared at 12 h p.i. and subjected to western blot analysis with anti-ORF8 antiserum. The estimated sizes of detected bands are indicated with arrowheads. Size markers are indicated on the left of the panels

Drosophila heat shock promoter inserted within *orf8*. A plasmid DNA containing the *orf8* disruption was co-transfected with BmNPV DNA into BmN cells and recombinant virus was isolated by identification of plaques expressing β-galactosidase. The first mutant was constructed so that the entire coding region of ORF8 was deleted (*Eco*R I to *Nhe* I, Fig. 4A). We were not able to obtain a viable recombinant virus lacking the entire ORF. Several attempts were made to plaque-purify this recombinant, but the blue plaques obtained were always contaminated with wild-type virus. This suggests that *orf8* may have an important role during viral infection.

Therefore, we constructed another plasmid with only the C-terminal portion of ORF8 deleted (*Bbs* I to *Nhe* I, Fig. 4A), and a disruption mutant was isolated, which we named BmD8. The disruption of *orf8* gene was confirmed by PCR and Southern hybridization (data not shown). We further performed western blot analysis using BmN cells infected with wt or BmD8. An immunoreactive band of 13 kDa was detected in BmD8 infected cells (Fig. 4B, lane 2). This size corresponds to the

predicted C-terminal truncation version of ORF8 since the calculated molecular mass is 12.8 kDa.

BV production and viral DNA replication in BmD8-infected cells

We then examined the effect of *orf8* disruption on BV production. BmN cells were infected with BmD8 or wt BmNPV, and yields of BV were determined by plaque assay. BmD8 showed a slower rate of BV production in BmN cells throughout infection, and this was more pronounced after 20 h p.i. (Fig. 5A). At 24 h p.i., the yield of BmD8 was only 1.7×10^7 PFU/ml while wt produced 8.6×10^7 PFU/ml. The final titer of BmD8 at 72 h p.i. was also 3.2-fold lower than that of wt (2.1×10^8 and 6.8×10^8 PFU/ml, respectively). This result indicates that the deletion of *orf8* gene impaired the production of progeny virus.

To determine whether the lower yields of virus were due to a decreased level of viral DNA replication, the accumulation of viral DNA during infection was monitored by dot blot analysis. The levels of viral DNA were similar for wt BmNPV and BmD8 mutant throughout infection (Fig. 5B). This suggests that the disruption of *orf8* gene did not affect the synthesis of viral DNA. To see whether the results are reproducible, we performed this experiment three times independently.

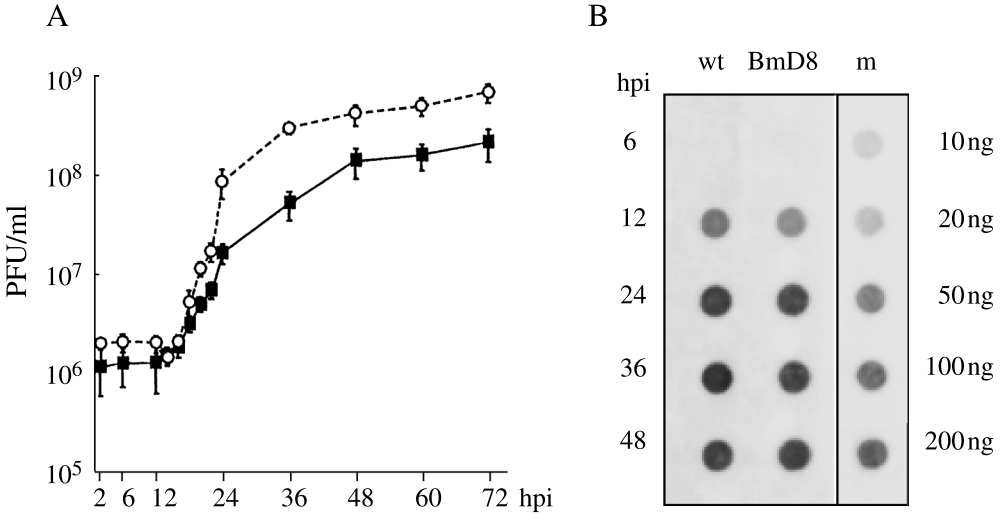


Fig. 5. Analyses of virus production and DNA replication in BmN cells. **A** Growth curves of wt BmNPV and BmD8 in BmN cells. BmN cells were infected with wt BmNPV (broken line with circles) or BmD8 (solid line with squares) at an MOI of 10 PFU per cell. At 2, 6, 12, 14, 16, 18, 20, 22, 24, 36, 48, 60, and 72 h p.i. (shown on the bottom), culture media were collected and subjected to plaque assay on BmN cells. The results represent the average of three independent experiments, and standard errors are indicated. **B** Dot blot analysis of wt and mutant viral DNA replication. Infected cells were harvested at indicated times p.i. (shown on the left). Dots on lane m represent purified BmNPV DNA (amounts are shown on the right) used as a standard. BmN cells were infected with wt or mutant virus at an MOI of 10 PFU per cell

Then, the quantified signals were compared between wt BmNPV and BmD8 on each infection time. The ratio of BmD8 to wt BmNPV were 1.016 ± 0.067 at 12 h p.i., 0.962 ± 0.055 at 24 h p.i., 0.981 ± 0.043 at 36 h p.i., and 0.986 ± 0.038 at 48 h p.i., confirming the reproducibility.

Binding ability of ORF8 protein to nucleic acids

We next investigated the ability of ORF8 to bind nucleic acids by using column chromatography on ssDNA-cellulose, dsDNA-cellulose or polyU-agarose. Extracts of BmNPV infected cells were loaded onto separate columns packed with each resin. Columns were washed and eluted with stepwise salt gradients. Proteins eluting at each step were subjected to western blot analysis using ORF8 antiserum (Fig. 6). The elution profile for ssDNA showed that the bulk of ORF8 was eluted at 0.7 M NaCl while the elution peaks for dsDNA and polyU were at 0.5 M NaCl. These results indicate that ORF8 bound nucleic acids or strongly interacted with a nucleic acid-binding protein, and that it interacted more strongly with ssDNA than dsDNA or synthetic RNA.

Analyses using antiserum against BV/ODV-E26 of AcNPV

We examined whether ORF8 was associated with virions of BmNPV because Ac16 (*da26* or *bv/odv-e26*) was reported to encode an envelope protein of BV and ODV [3]. However, we were not able to detect ORF8 either in BV or ODV

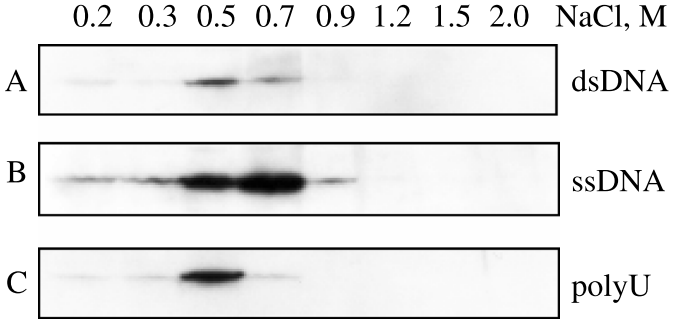


Fig. 6. Interaction of ORF8 protein with nucleic acid resins. BmN cell extracts were prepared from BmNPV infected cells at 12 h p.i. and subjected to dsDNA-cellulose **A**, ssDNA-cellulose **B** and polyU-agarose **C** column chromatography. The collected fractions at the indicated concentrations of NaCl were analyzed by Western blot analyses

Fig. 8. Double staining of ORF8 in BmNPV infected BmN cells using anti-ORF8 and anti-E26. Top and middle panels show the immunofluorescence images with anti-E26 and anti-ORF8, respectively. These were merged in bottom panels. BmN cells were mock infected or infected with BmNPV for 4, 8 and 12 h, and subjected to immunohistochemistry. The scale bar indicates 10 μm

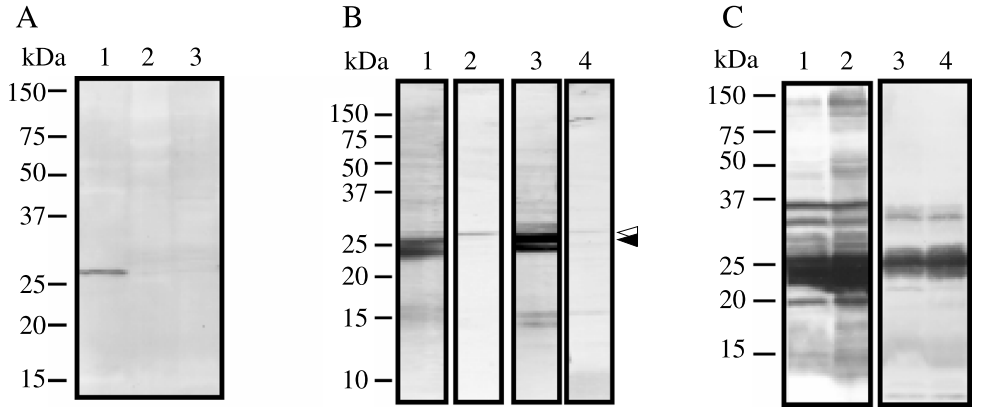
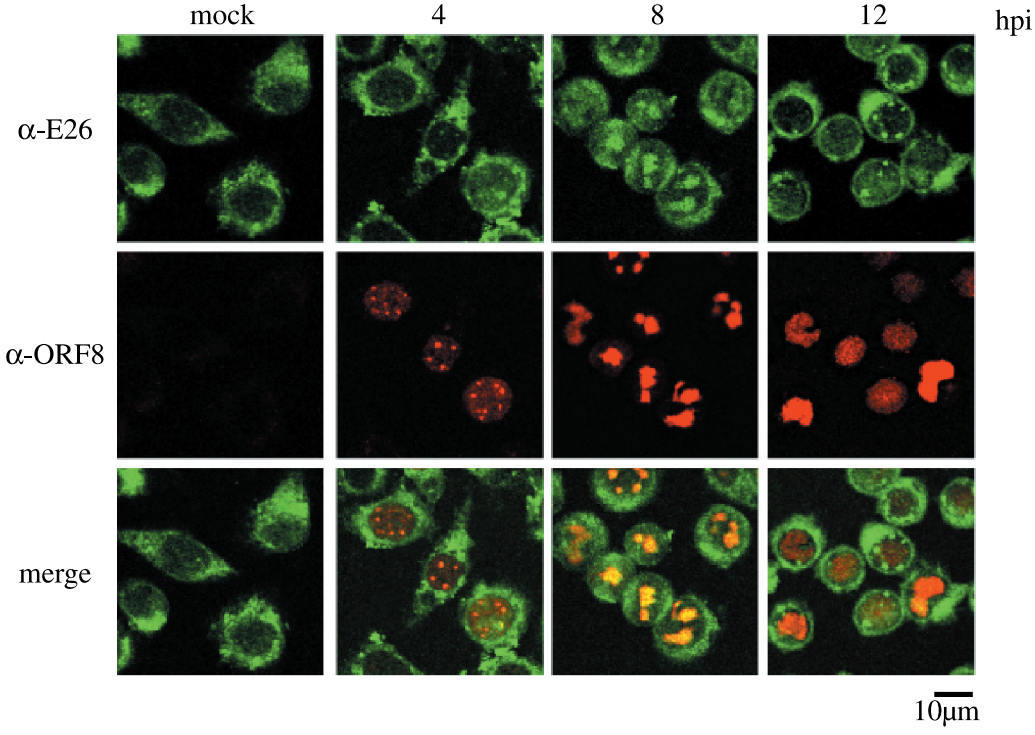


Fig. 7. Western analyses using anti-ORF8 and anti-E26. **A** Analysis using viral particles of BmNPV. Extracts of infected BmN cells at 12 h p.i. (*1*), purified BV (*2*, 14 μg) and ODV (*3*, 14 μg) were subjected to western analysis using anti-ORF8. Size markers are indicated on the left side of the panels. **B** Analysis using infected cells. Extracts of wt AcNPV-infected Sf9 cells (*1* and *2*) and wt BmNPV-infected BmN (*3* and *4*) were subjected to western analysis using anti-E26 (*1* and *3*) and anti-ORF8 (*2* and *4*). Open arrowheads indicate the polypeptide corresponding to ORF8 and closed ones show the major protein detected by anti-E26. **C** Comparison of viral particles from wt and BmD8 using anti-E26. Purified BVs (*1* and *2*) and ODVs (*3* and *4*) of wt BmNPV (*1* and *3*) and BmD8 (*2* and *4*) were subjected to western analysis using anti-E26. Fourteen μg of purified virions were loaded on each lane



(Fig. 7A). Since this conflicted with the results of Beniya et al. [3], antiserum prepared against BV/ODV-E26 of AcNPV (anti-E26) was tested on blots of AcNPV infected *Spodoptera frugiperda* Sf9 cells and BmNPV infected BmN cells. The anti-E26 detected ORF8 but reacted most strongly with a protein that was slightly smaller than ORF8 in both AcNPV-infected Sf cells and BmNPV-infected BmN cells (Fig. 7B, lanes 1 and 3). Reaction with anti-ORF8 in parallel revealed the presence of a single immunoreactive band in both extracts (Fig. 7B, lanes 2 and 4). Because anti-E26 crossreacted with other bands in addition to ORF8, we compared immunoblots of viral particles from wt BmNPV and BmD8 (Fig. 7C). Although numerous immunoreactive bands were observed with anti-E26, there was no difference in the pattern confirming that these proteins were not ORF8.

Although anti-E26 crossreacted with other cellular and viral proteins, western analysis using the nuclear fraction of BmNPV infected BmN cells showed a distinct immunoreactive band in nuclear fractions with the predicted size of ORF8 (data not shown). Therefore, the subcellular localization of ORF8 in BmNPV infected BmN cells was compared using anti-ORF8 and anti-E26. Beniya et al. have previously shown that BV/ODV-E26 was localized in punctate structures within the cytoplasm at 6 h p.i. [3], but at 16 h p.i. was distributed in both nuclear and cytoplasmic foci. However, our analysis revealed diffuse cytoplasmic staining as well as distinct nuclear foci from 4–12 h p.i (Fig. 8). The cytosolic staining was also observed with anti-E26 and mock-infected cells, indicating that it was non-specific, while the nuclear foci were infection-specific and corresponded to those sites labeled with anti-ORF8.

Discussion

In this report, we demonstrated that BmNPV *orf8* was expressed as an early gene, and that the encoded protein accumulated in the nucleus. We also showed that ORF8 colocalized with IE1 throughout infection.

It has been reported that transcription and replication of eukaryotic DNA viruses take place in specific regions of nucleus, which are known as ND10 [22]. Recent evidence from our lab and others suggest that insect cells also contain ND10 structures. Murges et al. showed that AcNPV IE2 associates with the insect homologue of one mammalian ND10 component, suggesting that ND10-like structures can be formed in insect cells [24]. Okano et al. reported that BmNPV IE1 localizes to specific foci in the nucleus [27]. In addition, at least two viral replication factors, DBP and LEF3, colocalize with IE1 during DNA replication [27]. Mainz et al. also showed replication factor DBP associated with AcNPV IE2 structures [21]. Together, these observations indicate that insect cells contain ND10 structures, and viral DNA replication proteins are closely related to these structures. Therefore, the colocalization of ORF8 with IE1 suggests that ORF8 may also play a role in viral DNA replication. We showed that ORF8 was colocalized with IE1 from 4 h p.i., before the onset of DNA replication, until 20 h p.i. which is after DNA replication ceases. Okano et al. noted that DBP was

not colocalized with IE1 either before or after the DNA replication while LEF3 colocalized with IE1 after DNA replication but not before the onset of replication [27]. Baculovirus IE1 is essential not only for viral DNA replication [15] but also for transcription of viral genes [30]. Thus it is also possible that ORF8 is involved in transcription, as previously suggested [7].

The ability of ORF8 to bind nucleic acids also supports a role for ORF8 in DNA replication or transcription. This is consistent with growth curves showing that BmD8 produces less progeny virus. Although our analysis showed that viral DNA replication was not affected by disruption of *orf8*, it is still possible that ORF8 plays a role in viral DNA replication because the N-terminus of ORF8 is expressed in BmD8-infected cells. Furthermore, it was not possible to knock out the *orf8* gene. Taken together with the finding that BmD8 produces a truncated version of the protein, it suggests that the missing portion of ORF8 contributes to its function.

The AcNPV homologue of *orf8*, *da26* (*bv/odv-e26*), was reported to encode an envelope protein of BV and ODV [3]. However, our western analysis using viral particles showed that BmNPV ORF8 was not a structural protein either of BV or ODV. Since this conflicted with the results previously obtained for the AcNPV homologue, we conducted further tests with anti-E26. We found that anti-E26 was able to detect ORF8 very weakly but strongly crossreacted with another protein slightly smaller than ORF8. Furthermore, anti-E26 failed to show any difference between BVs or ODVs of wt BmNPV and BmD8. These suggest that the viral envelope protein previously identified as BV/ODV-E26 is not encoded by Ac16, but is, instead, a slightly smaller protein encoded by another gene. The localization of ORF8 was also different from that previously reported with the AcNPV homologue [3]. Therefore, we compared the localization of ORF8 using anti-E26 and anti-ORF8. Anti-E26 strongly reacted with cytosolic proteins in mock infected cells, so we could not confirm the cytoplasmic localization. However, we found that the nuclear reactivity with anti-E26 colocalized with ORF8 foci through 12 h p.i.

O'Reilly et al. reported that the disruption of *da26* did not affect viral replication of AcNPV [26]. They disrupted DA26 by constructing a *lacZ* in-frame fusion following the 118th aa residue of DA26, suggesting that partial DA26 could be expressed in this recombinant virus. Other mutant viruses in which *da26* was disrupted by deletion and by insertion of transposable elements were isolated after serial passage of the virus in cell culture. One of these had an extended deletion from the *egt* gene (the N-terminal flanking gene of *da26*) into the N-terminus of *da26*, suggesting that *da26* would not be expressed in this, and possibly other of the insertion and deletion mutants [26]. In our studies, however, we were not able to isolate a null mutant. This could be due to the essential nature of *orf8* gene or to one of the adjacent genes. The N-terminal flanking gene is *orf7* (*egt*) and the C-terminal one is *orf9* whose function is unknown. Transcription of these three genes occurs in same direction, although we don't know whether they are transcribed as one transcript or not. The deleted C-terminal region of *orf8* in BmD8 might include the promoter for *orf9* since these two genes overlap

(Fig. 4A). However, BmD8, the C-terminal ORF8 deletion, which presumably also deletes the *orf9* promoter, was successfully isolated. In addition, it was possible to isolate an *orf9* deletion mutant (S. Gomi, personal communication). These results suggest that deletion of the entire region of *orf8* would not be deleterious because of effects on *orf9*. Together with the report that *egt* gene can be deleted in cell culture [11], we concluded that unsuccessful isolation of a null mutant was most likely due to a requirement for *orf8*, suggesting that BmNPV *orf8* is essential for viral replication. The differences between the BmNPV and AcNPV system imply that the homologues of BmNPV *orf8* may differ in their essential roles due to adaptation to different hosts. In support of this, it has been reported that a mutation in AcNPV *ie2* showed different effects on BV production, viral DNA replication and trans-regulatory activity in different host cells, such as SF21, TN-5B1-4, and TN368 [29]. In addition, Morishima et al. [23] suggested that P35 genes have evolved during baculovirus evolution because the anti-apoptotic activities of P35 from AcNPV and BmNPV were different.

In this report, we demonstrated that the *orf8* gene of BmNPV encodes a nuclear protein which may be involved in transcription and/or viral DNA replication. We also showed that BmNPV ORF8 is not associated with virions and suggested that *Ac16* may not encode an envelope protein (BV/ODV-E26). The function of OpNPV and EppoNPV homologues still remains unclear, therefore, the analysis of these genes should give more information regarding its function during viral infection.

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