Replication and Occlusion Body Formation of *Spodoptera exigua* Multicapsid Nucleopolyhedrovirus in a Homologous Cell Line

Sudawan Chaeychomsri
Central Laboratory and Greenhouse Complex, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
Email: rdisuc@ku.ac.th

Win Chaeychomsri and Jindawan Siruntawineti
Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
Email: {fsciwcc, fscijws}@ku.ac.th

Motoko Ikeda and Michihiro Kobayashi
Laboratory of Sericulture and Entomoresources, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan
Email: mochiko@agr.nagoya-u.ac.jp, michihir@nuagr1.agr.nagoya-u.ac.jp

***Abstract*—A continuous cell line, designated SENL1, has been established from *Spodoptera exigua*. The susceptibility of this cell line to its homologous virus, *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV), was evaluated on the basis of cytopathic effects, virus replication and morphogenesis in the infected cells. This cell line was highly susceptible to SeMNPV. By 3 days postinfection (pi), 99% of the cells contained occlusion bodies (OBs). Electron microscopy indicated that the OBs of SeMNPV produced in infected SENL1 cells were on average significantly larger than those produced in the infected larvae. Aberrant morphogenic characteristics such as abnormal OB formation and virion occlusion were observed in the SeMNPV-infected cells. A significant reduction in virions and nucleocapsids per OB was noted in the SeMNPV OBs produced in the infected cells when compared with the OBs produced in infected larvae. However, SeMNPV OBs obtained from infected SENL1 cells were infectious for *S. exigua* larvae, demonstrating that virus replication in vitro yielded viable progeny. The results from the present study suggest that the morphology and biological activity of SeMNPV OBs are influenced by some factors both in host cells and virus interactions. Thus, SENL1 cells may provide an in vitro system for studying possible cell effects on SeMNPV OB morphogenesis and ODV occlusion.**

***Index Terms*—*Spodoptera exigua*, nucleopolyhedrovirus, electron microscopy, virus morphogenesis, insect cell culture**

I. Introduction

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) is an important entomopathogenic virus belonging to the family Baculoviridae [1]. SeMNPV has a circular, double-stranded, supercoiled DNA genome of 135,611 bp, packaged in a rod-shaped nucleocapsid [2]. NPVs represent a group of DNA virus whose virions are embedded into polyhedron-shaped Occlusion Bodies (OBs) or polyhedra in the nuclei of host cells. During the infection process of NPVs, two distinct phenotypes, Occlusion-Derived Viruses (ODVs) and Budded Viruses (BVs) are produced [3]. ODVs are occluded in the polyhedrin matrix to form OB that protect the infectious virions from environmental degradation. ODVs are responsible for the establishment of the primary infection in insect midgut cells, whereas BVs are not occluded and responsible for the systemic spread of infection within the host larvae and also responsible for cell-to-cell transmission in culture.

SeMNPV has been developed and registered as a bioinsecticide for a number of years to control the beet armyworm (*S. exigua*), one of the most destructive polyphagous pests of a wide variety of crops worldwide [4]-[7]. The usefulness of SeMNPV in control of the beet armyworm has focused interest in selecting strains with high pathogenicity and genetic stability. Different strains of naturally occurring SeMNPV have been isolated from many geographical regions of the world, including The Netherlands [8], California [9], Spain [10], Florida [11], Japan [12], Thailand [13], China [14] and Mexico [15]. Molecular and biological characteristics of these isolates have been widely reported [2], [15]-[18]. SeMNPV differs significantly from other baculoviruses in that it is a highly pathogenic host-specific virus. A high degree of specificity and infectivity of SeMNPV against *S. exigua* both in vivo and in vitro has been demonstrated [9], [19]-[22].

Several cell lines from *S. exigua* have been established for the primary purpose of studying and optimizing the OBs production [23]. Previously, the KU-SENL-1

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(SENL1) cell line from the neonate larvae of *S. exigua* was successfully established and tested for permissiveness to *Autographa californica* MNPV (AcMNPV) [24]. In order to evaluate the potential of this cell line for the production of SeMNPV, the virus was propagated in cell monolayers and the kinetics of virus replication were determined. Ultrastructural studies on virus replication and morphogenesis in the infected cells of *S. exigua* were also described.

II. Materials and Methods

A. Cell Line

The SENL1 cell line [24] was grown as monolayers in 25-cm^2 tissue culture flasks. Cultures were maintained in TC100 medium [25] supplemented with 10% fetal bovine serum (FBS) and incubated at 27°C. The cells were subcultured at 5-day intervals.

B. Virus

The SeMNPV isolate used in this study was SeMNPV-K, which originated from naturally-infected *S. exigua* larvae in green onion fields in Nakhon Pathom province, Thailand. The virus was propagated in *S. exigua* larvae to generate infectious hemolymph containing BVs.

Fourth instar *S. exigua* larvae were allowed to feed individually on formalin-free artificial diet [26] that had been contaminated with OBs of SeMNPV at a concentration of 1 x 10^5 OB/cm^2 diet. Four days after inoculation, infectious hemolymph containing BVs was collected by bleeding the infected larvae from the first proleg. The hemolymph was transferred immediately into ice-cold, sterile microcentrifuge tubes containing L-cysteine to prevent melanization [27], pooled and diluted at a ratio of 1:2 with complete culture medium. The diluted infectious hemolymph was passed through a 0.45 µm membrane filter and used as an inoculum for infection of the SENL1 cells.

C. Virus Infection

Monolayers of the SENL1 cells in 25-cm^2 tissue culture flasks were mock-infected with culture medium or were infected with SeMNPV at a ratio of 1 ml/10^6 cells. The virus inoculum was allowed to adsorb, with gentle rocking, for 1 h at room temperature. At the end of the adsorption period, the inoculum was removed and then the cells were rinsed twice with culture medium without FBS. Time zero was defined as the time when the fresh medium was added to the cells and incubated at 27°C. The cultures were observed daily for evidence of cytopathic effects (CPEs). Culture media were collected at the designated times, and BV production was determined by plaque assay on SENL1 cells [24]. The purified OBs were suspended in sterile distilled water and stored at 4℃. Haemocytometry was used to determine the concentration of the OBs in the virus stock.

D. Scanning Electron Microscopy

The SeMNPV-infected cells were harvested by tapping the bottom of the flask to release attached cells, pelleted at 1,000g and washed twice in phosphate-buffered saline (PBS). Pellets were resuspended and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, for 2 h at 4℃, and washed twice with PBS. Samples were seeded onto aluminum stubs and were allowed to air dry. Then stubs were coated with gold and examined using the scanning electron microscope, Hitachi SU8020 at 5 kV.

E. Transmission Electron Microscopy

The SeMNPV-infected cells containing OBs were harvested, pelleted and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, for 2 h at 4℃. The samples were washed with phosphate buffer pH 7.2 at 4℃ and resuspended in 2% SeaPlaque agarose. The cell-agarose block was cut into small cubes of about 1-2 mm length and washed with phosphate buffer, before being fixed with 1% osmium tetroxide, for 1 h at 4℃. After washing, the samples were sequentially dehydrated in acetone, infiltrated and embedded in Epon resin. Samples were cut with ultramicrotome, stained with lead citrate and uranyl acetate and observed under a transmission electron microscope, Hitachi HT7700 at 80 kV. Counts were made of the average number of virions per OB, the average number of nucleocapsids per virion, and the percentage of singly embedded virions.

In order to compare in vitro and in vivo replication of the virus, SeMNPV-infected larvae were processed for electron microscopy. Third instar *S. exigua* larvae were fed on artificial diet without formalin [26] which was surface-contaminated with SeMNPV at a concentration of 1 x 10^5 OB/cm^2 diet. Three days after inoculation, the larvae were dissected, fixed, and processed as described above.

III. Results and Discussion

A. Virus Infection

In order to determine SeMNPV replication in SENL1 cells, the infectious hemolymph containing BVs was used as an inoculum. The results showed that the SENL1 cells were highly susceptible to SeMNPV. Typical CPEs like granulated and rounded cells, nuclear hypertrophy and impairment in cell proliferation were observed within 1 day pi. The CPEs developed rapidly with subsequent OB formation. The SeMNPV replicated in both epithelial-like cells and spindle-shaped cells and OBs were clearly seen within 1 day pi (Fig. 1). These cells became loosely attached to the substrate. By 2 days pi, approximately 70% of total cells had numerous OBs (Fig. 1). At 3 days pi, the numerous OBs were formed in the hypertrophied nuclei of infected cells and 99% of the cells contained OBs. Some of the infected cells were dislodged from the culture flask and OBs were occasionally observed in the cytoplasm, suggesting that the nuclear membrane had been disrupted (Fig. 1). At the late stage of infection (4 days pi), some infected cells ruptured, releasing OBs into the medium (Fig. 1). At 5 days pi, lysed cells, partially lysed cells, nuclei with OBs, and extracellular OBs were seen in the culture medium. These observations suggested that infection of SENL1 cells by SeMNPV was successfully completed.

To determine if the SENL1 cells were fully permissive and produced progeny virions, culture media were harvested at various times pi (0, 6, 12, 18, 24, 48, 72, 96 and 120 h). BV titration was performed by plaque assay [24]. All samples were collected from three separate experiments with assays performed in triplicate on SENL1 cells. The virus growth curve was constructed using the mean titer for each time point.

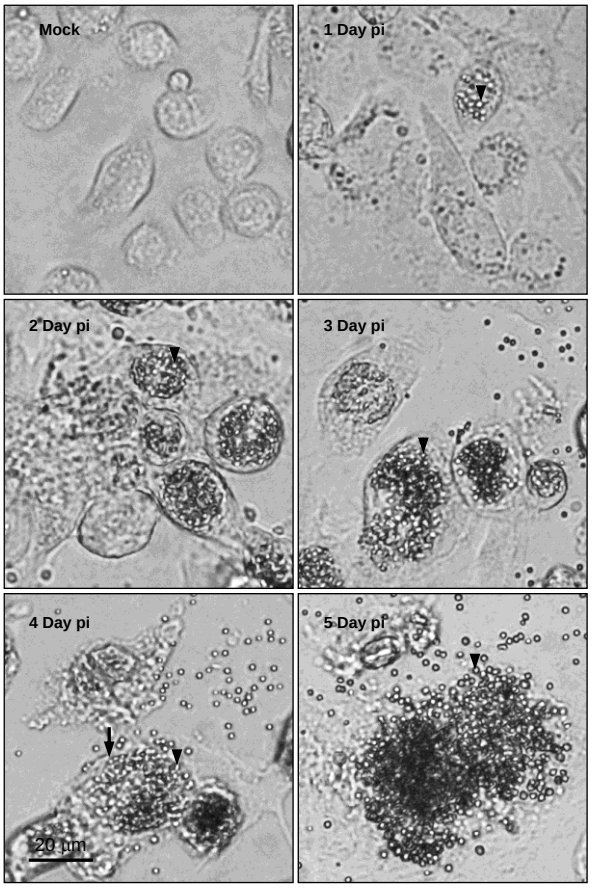

Figure 1. Cytopathology and OBs formation in SeMNPV-infected SENL1 cells. At 1-3 days pi, the infected epithelial-like cells and spindle-shaped cells were filled with OBs (arrowheads). At 4 days pi, the numerous OBs were formed in the hypertrophied nuclei of infected cells and some cells ruptured (arrow). At 5 days pi, almost infected cells lysed and released OBs into culture medium.

The titers of infectious BV from SeMNPV-infected SENL1 cells at various times pi are shown in Fig. 2. For the first 6 h pi, only the residual virus inoculum could be detected. At 12 h pi, there was an increase in BV titer which continued until reaching peak levels of 1.0×10^6 PFU/ml at 72 h pi and started to plateau thereafter.

B. Scanning Electron Microscopy

Light microscopy showed that infection of SENL1 cells with SeMNPV caused cell lysis. At 5 days pi, almost all OBs were released from lysed cells into culture medium (Fig. 1). Limitations of light microscopy for the study of CPEs of virus infection in host cells precluded observation of three-dimensional changes in OB morphology which occurred during SeMNPV infection. In the present study, the superior resolution of SEM was used to investigate this phenomenon. SEM observations of SeMNPV OBs revealed that they were roughly spherical in shape (Fig. 3A-C), with variable diameters ranging from 1.14 to 5.0 µm. The diameter of a completely formed OB was found to be 1.86 ± 0.62 µm (mean ± SD), whereas OBs prepared from dead larvae had diameters between 0.6 and 2.29 µm with an average value of 1.26 ± 0.37 µm (n = 50) (Fig. 3D). Thus, the OBs of SeMNPV produced in infected SENL1 cells were significantly larger than those produced in the infected larvae (analysis of variance, $P<0.001$).

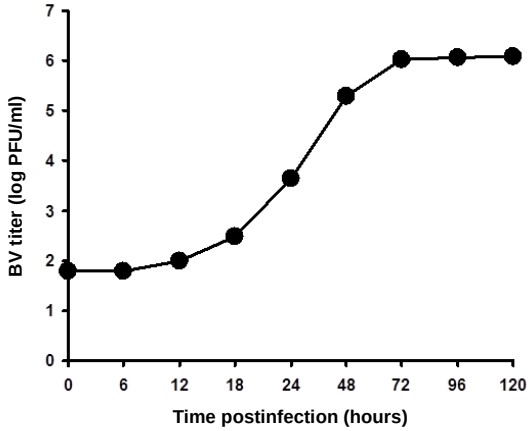

Figure 2. BV yields in culture medium from the SeMNPV-infected SENL1 cells. The titer of virus was determined by plaque assay.

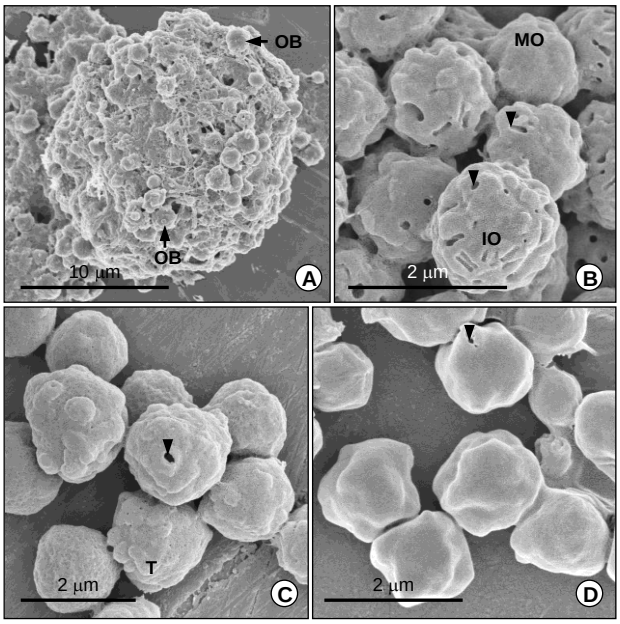

Figure 3. Scanning electron micrographs of the OBs of SeMNPV. (A) Ruptured cells showing clusters of OBs. (B) Immature OBs (IO) showing pitted surfaces and mature OBs (MO) possessing relatively smooth surface. (C) OBs released from infected SENL1 cells. (D) OBs obtained from dead larvae. Arrowheads indicate empty spaces left by dislodged virions. T, triangular OBs.

Scanning electron micrographs of OBs obtained from in vitro-produced SeMNPV also revealed an OB shape that was roughly triangular (Fig. 3C). The faces of the triangles were usually not uniform and bulbous protrusions were observed. During the last phase of the infection cycle, almost infected cells lysed and released

OBs into culture medium. At that time immature as well as mature OBs appeared (Fig. 3B). The immature OBs showing pitted surfaces, while the mature OBs possessing smooth surface were observed under SEM (Fig. 3). Some of the OBs obtained from both in vitro- and in vivo-produced SeMNPV had their virions dislodged from the polyhedrin matrix, leaving empty spaces and holes in the OBs. Mature OBs are surrounded by an electron-dense structure called the Polyhedron Envelope (PE) [28]. The Polyhedron Envelope Protein (PEP) has been shown to play a major role in sealing the periphery of the OBs, thereby ensuring retention of nucleocapsids that may otherwise become dislodged and lost [28]-[32].

C. Transmission Electron Microscopy

To further study whether the in vitro condition has any effect on virus morphogenesis, transmission electron microscopy was performed with ultrathin sections generated from SENL1 cells infected with SeMNPV (Fig. 4). The infected cells exhibited the typical baculovirus infection with all the nuclei approximately in the same stage of virogenesis. Virogenic stroma observed in the enlarged nuclei appeared as a loose granular material, located centrally and surrounded by rod-shaped nucleocapsids at different stages of envelopment and occlusion (Fig. 4A). Nucleocapsids were partially or completely enclosed either singly or multiply within an envelope and embedded within a crystalline matrix of polyhedrin forming a polyhedral inclusion body or OB. These virions (enveloped nucleocapsids) were scattered throughout the nuclei, and a few developing OBs were seen in the infected cells (Fig. 4A). During this stage of infection, virions were occluded within OBs. Late in infection, the immature and mature OBs with embedded virions and the PE were abundant in the infected cells (Fig. 4B, C). Mature OBs were entirely surrounded by the PE, while the immature OBs were partially wrapped by PE (Fig. 4C, D).

In third instar larvae of *S. exigua* infected with SeMNPV OBs prepared from dead larvae, virtually all cells of the fat body were infected by 3 days postinoculation. Characteristically, infected cells had greatly enlarged nuclei with a properly formed virogenic stroma. Both naked and enveloped nucleocapsids could be seen within and around the electron-dense region of the virogenic stroma. Large numbers of nucleocapsids accumulated in the infected nuclei and aggregated into paracrystalline arrays. (Fig. 5A). When viewed in longitudinal, cross-sectional and oblique profiles of these well organized aggregates, these structures appeared as single or multiple tiers of nucleocapsids aligned in parallel. Nucleocapsids were enveloped singly or in groups and the enveloped nucleocapsids occluded by developing OBs (Fig. 5B, C). Relatively large numbers of OBs were produced and the mature OBs with entirely wrapped by PE contained numerous virions (Fig. 5D).

To further analyze if some factors from host cells had any effect on virus replication and morphogenesis, ultrathin sections of these specimens were examined more by TEM. Fifty cross-sectioned OBs of SeMNPV produced in the infected SENL1 cells and OBs produced in infected larvae were randomly chosen and visualized through electron microscopy to assess levels of virion occlusion. Counts were made of the average number of nucleocapsids per virion, the average number of virions and nucleocapsids per OB, and the percentage of singly embedded virions.

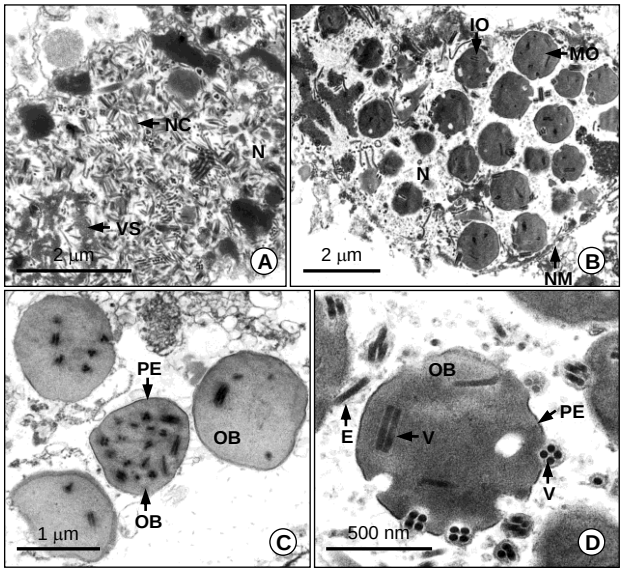

Figure 4. Transmission electron micrographs of SeMNPV-infected SENL1 cells. (A) Nucleocapsids and virions within and around a dense virogenic stroma. (B) Enlarged nucleus containing several OBs with embedded virions. (C) OBs with embedded virions. (D) OB containing virion bundles, and surrounded by discontinuous PE. E, envelope; IO, immature OB; MO, mature OB; N, nucleus; NC, nucleocapsid; NM, nuclear membrane; OB, occlusion body; PE, polyhedron envelope; V, virion; VS, virogenic stroma.

Examinations of sectioned OBs of SeMNPV produced in the infected cells revealed that each envelope contained 1 to 5 nucleocapsids, whereas OBs from infected larvae contained 1-6 nucleocapsids. The mean number and standard deviation of nucleocapsids per envelope of SeMNPV from cell and larva sources were 1.82 ± 1.01 and 1.87 ± 1.15 nucleocapsids, respectively, which were not statistically different. However, there were significant differences in the number of virions and the number of nucleocapsids per OB between the OBs of SeMNPV produced in the infected cells and OBs produced in infected larvae. When SeMNPV was propagated in SENL1 cells there was a significantly lower mean of virions and nucleocapsids per OB than after the virus had been propagated in larvae (analysis of variance, $P<0.001$). The mean number and standard deviation of virions per OB of SeMNPV from cell and larva sources were 4.20 ± 2.81 and 10.54 ± 5.25 virions, whereas the mean number and standard deviation of nucleocapsids per OB were 6.78 ± 5.25 and 19.74 ± 11.48 nucleocapsids, respectively. In SeMNPV-infected cells, there were 51.4% single-, 24.3% double-, and 24.3% multiple-nucleocapsid ODVs and 53.5% single-, 19.9% double-, and 26.6% multiple-nucleocapsid ODVs in SeMNPV from larva source. Although no significant difference was observed in the percentage of single- and multiple-nucleocapsid ODVs between the two viruses,

the percentage of single virion and single nucleocapsid per OB was significantly higher in SeMNPV OBs formed in the infected cells, when compared with SeMNPV OBs produced in the infected larvae. The high percentage (up to 16%) of OBs containing a single virion was observed in SeMNPV OBs formed in the infected cells, whereas the OBs containing a single virion was not observed in the infected larvae. Similarly, percentage of OBs containing a single nucleocapsid increased from 0 to 16% in OBs from infected cells when compared with the OBs produced in infected larvae. The number of nucleocapsid per OB of in vitro-and in vivo-produced SeMNPV varied and ranged from 1 to 20 and 3 to 43 nucleocapsids, respectively.

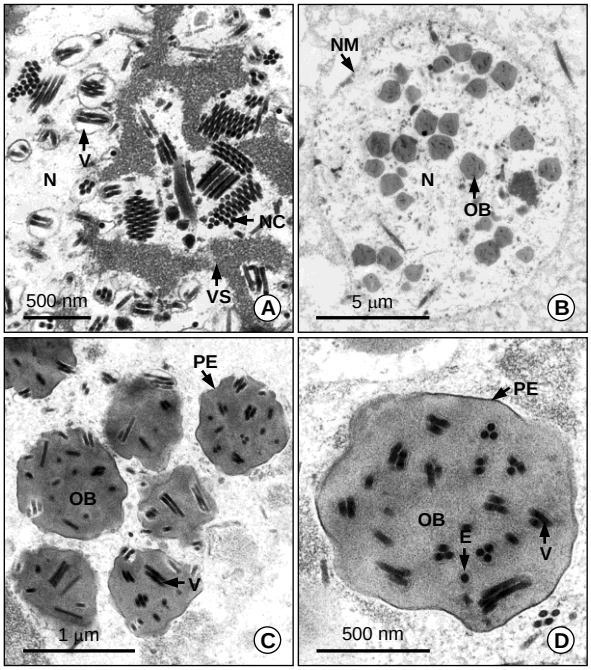

Figure 5. Transmission electron micrographs of SeMNPV infection in *S. exigua* larvae. (A) Nucleocapsids and virions within and around virogenic stroma. (B) Enlarged nucleus containing several OBs with embedded virions. (C) Virions in the process of being occluded into developing OBs. (D) Mature OB containing several virions. E, envelope; N, nucleus; NC, nucleocapsid; NM, nuclear membrane; OB, occlusion body; PE, polyhedron envelope; V, virion; VS, virogenic stroma.

As shown in Fig. 3, SeMNPV produced characteristic triangle-like OBs in the infected cells. Additionally, the occurrence of OBs that had very few virions was detected in some nuclei (Fig. 4C). These results indicated that there could be a defect in OB morphogenesis in SENL1 cells infected with SeMNPV. To further determine if some factors from host cells affect OB structure, sectioned OBs of SeMNPV produced in the infected cells were examined more by TEM. Aberrant virus morphogenic features such as abnormal OB formation and virion occlusion were observed (Fig. 6). The aberrant OBs produced by SeMNPV contained several, a few, very rarely and no virions.

Numerous cell lines from a variety of tissues of *S. exigua* have been documented [23]. These cell lines exhibit different susceptibility to virus and produce variable amounts of OBs [19]-[23], [33], [34]. Insect cell lines are classified into three types based on their permissiveness to support baculovirus replication. Various criteria have been used for the definition of the cell permissiveness [35], [36].

The results and observations obtained in this study strongly indicated that SeMNPV can replicate and produce infectious progeny virus in a homologous insect host, SENL1 cell line. Therefore, the SENL1 cells were fully permissive for SeMNPV infection.

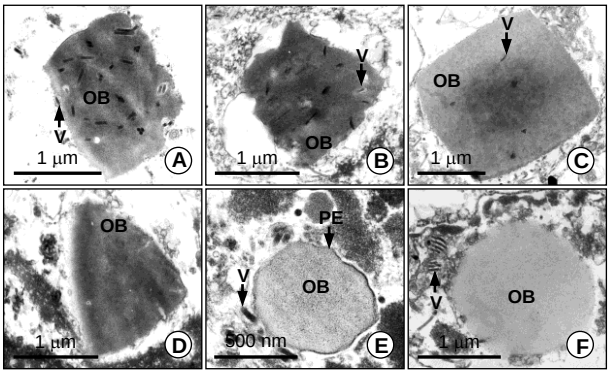

Figure 6. Transmission electron micrographs of OBs with aberrant morphology in the nucleus of SeMNPV-infected SENL1 cells. (A) Irregular-shaped OB containing several virions. (B) Irregular-shaped OB containing only a few virions. (C) Cuboidal OB containing a single virion. (D) Triangular OB with no virion occlusion. (E) Virions surrounding empty OB. (F) OB with no virion occlusion and no PE. OB, occlusion body; PE, polyhedron envelope; V, virion.

The criteria for SeMNPV replication in this permissive cell line were based on: (a) cytopathology, in which SeMNPV-infected SENL1 cells did produce typical CPEs characteristic of NPV infections, (b) replication kinetics, which revealed the measurable amounts of infectious progeny virus, (c) scanning electron microscopy, which revealed the presence of OBs formed in the infected cells, and (d) ultrastructural study, which displayed the morphological sequence of NPV infection. Thus, infection of SeMNPV in SENL1 cells was successfully completed as in vitro.

Light and electron microscopic observations revealed that SeMNPV replicated well in the SENL1 cells and exhibited the many polyhedra (MP) phenotype (based on a large number of OBs produced per cell). The SeMNPV MP plaque phenotype was retained and accumulation of few polyhedra (FP) plaque was not detected after in vitro passage. This is supported in part by the observation that individual plaques obtained from progeny virus (passage 1) exhibited MP characteristics (Fig. 7A).

Interestingly, the size of SeMNPV OBs produced in the infected cells was 47% larger in diameter than those of the OBs derived from infected larvae when examined by SEM. The OBs of SeMNPV produced in different cell lines tend to differ in their sizes. The OBs of SeMNPV produced in SENL1 cells were larger than those produced in UCR-SE-1 cells, which averaged 1.64 ± 0.16 μm [19]. It has been suggested that there is a host cell factor that is responsible for the determination of OB size [37].

SEM observations of SeMNPV OBs obtained from both in vitro-and in vivo-produced revealed that OBs produced in SENL1 cells comprised about 40% of

immature OBs while OBs prepared from dead larvae comprised less than 5% of immature OBs (Fig. 3), which confirmed that the morphology and maturation of SeMNPV OBs were controlled not only by the viral genes but also by the host cell factors.

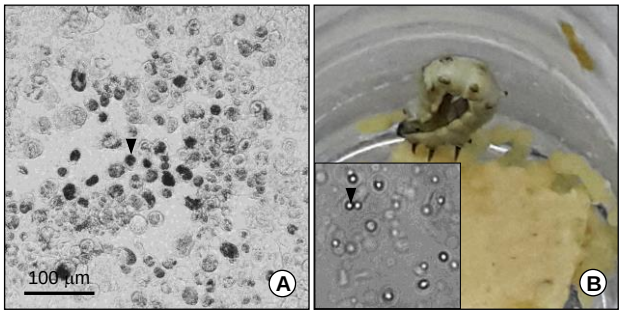

Figure 7. Biological activity of progeny virus harvested from SENL1 cells infected with SeMNPV. (A) MP plaque formed by BVs in SENL1 cell monolayers. Plaque consisting of numerous cells containing OBs (arrowheads) at 5 days pi. (B) Dead *S. exigua* larvae killed by the infectious OBs. Inset: crude suspension obtained directly from dead larvae showing the presence of OBs (arrowheads).

The PE structure is required for the maturation of OBs [28]. It is possible that improperly formed PEP can cause the apparent lack of PE and discontinuous PE in the infected cells (Fig. 4D). A cross-section of holes or hollows in the OBs indicated that they lack an electron-dense layer, which would be indicative of PE. These holes seem to be the empty spaces, as shown in Fig. 3, which are devoid of PE, indicating that the PEP failed to be properly associated with OBs. A hole as seen in an ultrathin section (Fig. 4D) is not an electron microscopy artifact during sample preparation, but it is an empty space on the OB surface (Fig. 3B) which is seen in different orientation. Both SEM and TEM observations indicate the presence of PE surrounding the OBs produced in the infected cells (Fig. 3, 4). However, these OBs had a rough surface with some bulbous protrusions (Fig. 3C), whereas OBs produced in infected larvae had a relatively smooth surface and sharp edges when examined under SEM (Fig. 3D). The protrusions observed in the in vitro-produced OBs are likely to be caused by the projections of the virus bundles during the occlusion phase. In the present study, the SEM analysis of mature OBs indicated their surface heterogeneity within the population, smooth and rough surface with some bulbous protrusions. This heterogeneity in surface structure within a population of OBs is due to the sources of OBs (in vitro- or in vivo-produced OBs).

Because the SeMNPV OBs produced in the infected cells were larger than OBs prepared from dead larvae, it might be expected that the number of virions per OB would be higher. However, SeMNPV OBs produced in the infected cells exhibited a significant reduction in virions and nucleocapsids per OB (Fig. 4, 5). The previous studies have demonstrated that the multiple-nucleocapsid ODVs are more efficient at establishing systemic infections due to a higher number of nucleocapsids per virion or "replicative units" [38]. Each ODV is theoretically capable of infecting a host midgut cell, resulting in progressive infection of the larvae [39]. Thus, the availability of virions in OBs might play a significant role during infection of *S. exigua* larvae. Based on the data from electron microscopy obtained in the present study, it was proposed that the decreased amount of ODVs in OBs would lead to lower oral infectivity or no oral infectivity at all when the larvae were fed with SeMNPV OBs produced in the infected cells. Interestingly, SeMNPV OBs produced in the SeMNPV-infected SENL1 cells were infectious for *S. exigua* larvae (Fig. 7B), indicating that defects in ODV or OB morphogenesis were not involved in the loss of in vivo activity of SeMNPV. The results reported here, obtained by using OBs from SENL1 cells, support earlier reports that OBs produced in Se301 cells are infectious in vivo [40]. It has been reported that OBs of SeMNPV isolate 608 produced in UCR-SE-1 cells are also infectious for *S. exigua* larvae [19], whereas OBs of SeMNPV/US produced in UCR-SE-1 cells are noninfectious per os [41]. Therefore, there may be differences in the induction of SeMNPV mutant viruses among the cell lines. A possible explanation for this phenomenon is that host cell factors may interact either directly or indirectly with the viral factors, resulting in reduced yield of ODVs and decreased infectivity.

It has been reported that the passage of SeMNPV in *S. exigua* cell lines leads to the rapid accumulation of deletion mutants, some of which have been demonstrated to have defective interfering (DI) properties (41], [42]. The OBs of these mutants are significantly less virulent in *S. exigua* larvae. This phenomenon is due to a deletion of about 25 kbp from the SeMNPV genome. Moreover, the genotypic alterations can result in changes in phenotypic characteristics, including virus morphology and biological activity. The occurrence and accumulation of defective or mutant viruses upon passage of NPVs in cell culture have been reported in several studies. The most common mutants are FP and DI particles. Both mutant viruses cause significant reduction in OB formation and virus infectivity [43]. Replication of DI particles rely on helper virus, while FP mutants do not need another virus to complete their infection cycle. The results from the present study indicate that SeMNPV mutants rapidly arise after only one passage in cell culture. Changes in OB morphogenesis are in agreement with the previous findings, in which the passage of SeMNPV in *S. exigua* cell lines leads to the rapid accumulation of DI mutants [41]. It has been reported that the accumulation of DI mutants with major deletions is a general phenomenon. DI mutants are present in the hemolymph-derived BVs (passage zero) collected from the infected *S. exigua* larvae and subsequently accumulated in cell culture. This is evidenced by the rapid generation of DI mutants within the first round of infection in SENL1 cells. This finding is likely similar to that seen for wild-type SeMNPV infecting the Se301 cells [44].

As seen by TEM, abnormal OBs were found with few or no virions and many virions were not occluded in the later stages of infection (Fig. 6). This observation indicated that although SeMNPV propagated in homologous cell line might be able to affect OB

morphogenesis. Similar abnormalities have also been observed in *Malacosoma disstria* cells infected with *Lambdina fiscellaria somniaria* NPV [45] and *S. frugiperda* cells infected with *Trichoplusia ni* NPV [46]. These reports suggest that malformation of OBs may be due to replication in the heterologous hosts rather than in the native host. However, the results of the current study showed that a defect in OB formation with few or no virions was observed in the SeMNPV-infected SENL1 cells (Fig. 6). These aberrant forms are similar to those observed in *S. frugiperda* cells infected with *S. frugiperda* NPV (SfNPV) [47] and *T. ni* (TN-368) cells infected with *T. ni* NPV [48]. The abnormalities are typical of the FP phenotype [49]-[52]. The phenotypic changes in the SeMNPV OBs observed in this study are likely to be the result of the persistence of DI mutants within the uncloned virus population (field isolate). This is because the FP plaque was not detected after in vitro passage as mentioned above. It may be theoretically possible that DI mutants lack one or more essential genes and therefore cannot form plaques initiated by an individual particle in such plaque assays, whereas FP mutants are able to form plaques.

Several reports have described a genetic alteration in in vitro-produced SeMNPV, while no major studies have been undertaken to elucidate the effect of in vitro propagation on the phenotype of these SeMNPV by electron microscopy. The present work reports detailed ultrastructural studies on the replication of SeMNPV in a homologous insect host, SENL1 cell line. The fast generation of the mutant viruses after only one passage in SENL1 cells is evidently not due to the effects of serial passage. This finding is similar to that observed in other NPVs as shown in the literature by studies in which abnormal OB formation and virion occlusion occurred in the infected cells upon the disruption of viral genes [53]-[57]. Therefore, the phenotypic changes in the SeMNPV OBs observed in this study are likely due to the influence of viral genes, thus limiting the nucleocapsid organization during ODV and OB assembly and occlusion.

Taken together, the results from the present study show that changes in phenotypic characteristics of SeMNPV can be affected by host as well as viral factors.

IV. Conclusions

The present study provides more informations on the in vitro propagation of the SeMNPV in homologous cell line and the abnormalities in virion envelopment and occlusion within OBs, leading to significant reduction in the assembly of virions. The findings in the present study suggest that the size of OBs and virus morphogenic features resulted from some factors both in host cells and in the viral genomes. These factors may play an important structural or accessory role in determining the size and shape of OBs, nucleocapsid assembly and ODV formation. Further studies comparing uncloned and plaque-purified isolates may provide more insight into the occurrence of aberrant morphology of OBs and, more generally, into the molecular basis of SeMNPV OB morphogenesis. In addition, isolation and testing of novel *S. exigua* cell lines are required, and may serve as beneficial tools for the propagation of SeMNPV that will optimize the use of baculovirus as a highly effective and highly specific insecticide.

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Sudawan Chaeychomsri received Doctoral Degree in Agricultural Science (Insect Virus Molecular Biology) from Nagoya University, Japan. Her current research interests focus on insect cell culture and insect virus molecular biology.

Win Chaeychomsri received Doctoral Degree in Agricultural Biotechnology (Animal Biotechnology) from Kasetsart University, Thailand. He is an outstanding lecturer in research and innovation. His current research interests focus on parasitology, immunology and animal biotechnology.

Jindawan Siruntawineti received Doctoral Degree in Agricultural Science from University of Tsukuba, Japan. Her current research interests focus on applied biochemistry, cell and molecular biology, immunology, and animal physiology.

Motoko Ikeda received Doctoral Degree in Agriculture (Sericultural Science) from Nagoya University, Japan. Her current research interests focus on insect virus molecular biology.

Michihiro Kobayashi received Doctoral Degree in Agriculture (Sericultural Science) from Nagoya University, Japan. His current research interests focus on insect virus molecular biology.