REPLICATION OF SPODOPTERA EXIGUA NUCLEOPOLYHEDROVIRUS IN PERMISSIVE AND NON-PERMISSIVE LEPIDOPTERAN CELL LINES

T. YANASE, C. YASUNAGA, T. KAWARABATA*

Institute of Biological Control, Faculty of Agriculture, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan

Received August 14, 1998; accepted September 1, 1998

Summary. – The Spodoptera exigua multinucleocapsid nucleopolyhedrovirus (SeMNPV) was inoculated to eight lepidopteran cell lines derived from *Spodoptera exigua* (Se301), *Spodoptera frugiperda* (SF21AEII), *Spodoptera littoralis* (CLS-79), *Spodoptera litura* (SpLi-221), *Pseudaletia separata* (LeSe-11), *Trichoplusia ni* (hi-5), *Plutella xylostella* (PXL/C) and *Bombyx mori* (BmN4). The productive infection of SeMNPV was observed only in Se301 cells. However, a dot-blot hybridization analysis revealed that SeMNPV DNA replicated in five non-permissive cell lines: SF21AEII, CLS-79, SpLi-221, hi-5 and BmN4. In addtion, the virus-infected hi-5 and BmN4 cells displayed morphological changes. In contrast, CLS-79 cells inoculated with SeMNPV showed membrane blebbing at 20 hrs post inoculation (p.i.) and fragmentation of genomic DNA. All that indicated that the infected CLS-79 cells underwent apoptosis. These findings indicate that the SeMNPV replication was restricted at various points in dependence upon each cell line.

Key words: Spodoptera exigua; baculovirus; nuclear polyhedrosis virus; host specificity; apoptosis; insect cell line

Introduction

Baculoviridae is a family of large, enveloped DNA viruses whose nucleocapsids contain a single molecule of circular supercoiled double-stranded DNA of 90 – 180 kbp (Murphy *et al.*, 1995). The members of this family have been isolated only from arthropods, primarily from insects including many serious pests. Thus, baculoviruses have been shown to be important biological insecticides in the control of pest populations. Usually, their host range is limited to a single species or several closely related species. Although they are harmless to non-target animals including beneficial insects and vertebrates, their limited host range makes them economically less attractive as compared to insecticides with a broad spectrum (Vlak, 1993; Thiem, 1997).

The family of *Baculoviridae* contains two genera, nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). Many insect cell lines have been established for the propagation of NPVs and investigated for the viral interaction with insect cells (Blissard, 1996). The Autographa californica multinucleocapsid nucleopolyhedrovirus (AcMNPV), which is the best characterized baculovirus, has been reported for the viral interaction with insect cell lines (McIntosh and Grasela, 1994; Blissard, 1996; Thiem, 1997). Although most of NPVs can replicate efficiently only in homologous insects or cell lines, AcMNPV has a wide host range; it reportedly infects members of 12 families of Lepidoptera and cell lines from 16 insect species (McIntosh and Grasela, 1993). In addition, AcMNPV is able to enter non-permissive insect cell lines and a substantial AcMNPV DNA replication proceeds in some of them (Morris and

*Corresponding author.

Abbreviations: AcMNPV = Autographa californica multinucleocapsid NPV; BmNPV = Bombyx mori NPV; DTA = ethylenediamine tetraacetate; FBS = foetal bovine serum; GV = granulovirus; MOI = multiplicity of infection; NOV = non-occluded virus; NPV = nucleopolyhedrovirus; p.i. = post inoculation; PIB = polyhedral inclusion body; SDS = sodium dodecyl sulfate; SeMNPV = Spodoptera exigua multinucleocapsid NPV

Miller, 1993). Moreover, reporter genes under control of AcMNPV promoters can be expressed in non-permissive cell lines (Morris and Miller, 1992, 1993). It has been shown that AcMNPV infection induces apoptosis in *Spodoptera littoralis* (SL2) and *Choristoneura fumiferana* (FPMI-CF-203) cell lines (Chejanovsky and Gershburg, 1995; Palli *et al.*, 1996). The shutdown of viral and cellular protein synthesis occurred in AcMNPV-infected *Lymantria dispar* (IPLB-Ld652Y and IPLB-LdFB) and *Bombyx mori* (BmN) cells at early time (Guzo *et al.*, 1992; Kamita and Maeda, 1993). These findings indicate that the cellular responses restrict the AcMNPV replication in each non-permissive cell line in a distinct manner. In contrast to AcMNPV, the host specificity of other baculoviruses has been reported poorly.

SeMNPV naturally infects the beet armyworm *S. exigua* that is a serious pest of agricultural crops in tropical and sub-tropical regions, and in greenhouse. Because SeMNPV is more virulent for *S. exigua* larvae than other baculoviruses, it has been tested in suppressing the *S. exigua* population (Gelernder *et al.*, 1986; Smits *et al.*, 1987). Previous studies on its *in vivo* host range have shown that only *S. exigua* larvae were susceptible to SeMNPV (Vlak *et al.*, 1981; Gelernder and Federici, 1986). Recently, Hara *et al.* (1994) reported that the formation of polyhedral inclusion bodies (PIBs) was observed only in *S. exigua* cell lines when SeMNPV was examined in 20 different cell lines from 8 different lepidopteran insects. However, there are only a few detailed studies about the host specificity of SeMNPV *in vitro*. Thus, in this study, we examined the replication of SeMNPV in permissive and non-permissive cell lines.

Materials and Methods

Cells and virus. The cell lines used in this study are listed in Table 1. The hi-5 cell line was cultured in EX-CELL™ 405 Medium (JRH Biosciences,USA) supplemented with 10% of heat-inactivated foetal bovine serum (FBS) at 27°C. The seven other cell lines were grown in IPL-41 Medium supplemented with 10% of heat-inactivated FBS at 27°C. The plaque-purified strain I1 of SeMNPV was used as a wild-type virus and propagated in *S. exigua* Se301 cells (Hara *et al.*, 1995). The virus samples were titrated as described by Hara *et al.* (1995).

Inoculation of cell lines with SeMNPV. Each cell line was seeded (2.0×10^5 cells/well) in a 24-multiwell tissue culture plate (Falcon 3047, Becton Dickinson, USA). Plates were centrifuged at 1,800 rpm (Sakuma R90-23 centrifuge, HM-2 rotor) for 15 mins to enhance the cell attachment to the well bottom and inoculated with 0.2 ml of virus inoculum per well at multiplicity of infection (MOI) of 5 PFU/cell. After incubation for 1 hr at 27°C, removal of the inoculum and washing with 0.5 ml of fresh medium per well, the infected cells were incubated with 0.5 ml of fresh medium per well at 27°C and examined under a light microscope. At certain intervals p.i., the cells were collected and stored at -80°C until use.

Table. Insect cell lines utilized in SeMNPV infection experiments

Species	Designation	Primary explant	Source
S. exigua	Se301	Neonate larvae	K. Hara[a]
S. frugiperda	IPLB-SF21AEII (SF21AEII)	Pupal ovaries	J. Vaughn[b]
S. littoralis	CLS-92	Pupal ovaries	J. Vaughn[b]
S. litura	TUAT-SpLi221 (SpLi-221)	Pupal ovaries	J. Mitsuhashi[c]
P. separata	NIAS-LeSe-11 (LeSe-11)	Fat body	J. Mitsuhashi[c]
P. xylostella	PXL/C	Neonate larvae	T. Sato[d]
T. ni	hi-5	Emryos	S. Maeda[e]
B. mori	BmN4	Emryos	Y. Hashimoto[f]

[a]Faculty of Agriculture, Kyushu University, Fukuoka, Japan.
[b]U.S. Department of Agriculture, Insect Pathology Laboratory, Beltsville, MD, USA.
[c]Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan.
[d]National Institute of Sericultural and Entomological Science, Ibaraki, Japan.
[e]Faculty of Agriculture, Tottori University, Tottori, Japan.
[f]Department of Applied Biology, Kyoto Institute of Technology, Kyoto, Japan.

Dot blot hybridization. To determine viral DNA replication, a dot blot hybridization was done as described previously with some modifications (Morris and Miller, 1993). Briefly, a cultured suspension containing 2.0×10^4 cells was centrifuged at 15,000 rpm (Tomy MX-160 centrifuge, TMA-24 rotor) for 20 mins and the pellet was resuspended in 100 µl of distilled water. The suspension was mixed with 81 µl of saturated NaI solution, incubated at 100°C for 15 mins and blotted to a nylon membrane (Hybond-N+, Amersham) by use of a dot blot apparatus (Atto, Japan). SeMNPV DNA was isolated from PIBs according to the procedure described by Hashimoto *et al.* (1994). To prepare a hybridization probe, SeMNPV DNA was digested with *Eco*RI and *Pst*I, and labelled with digoxigenin using a DIG DNA Labelling Kit (Boehringer Mannheim). Southern blot hybridization was carried out at 68°C overnight in a hybridization solution consisting of 5 x SSC, 1% Blocking Reagent (Boehringer Mannheim), 50 mmol/l sodium phosphate buffer pH 7.0, 7 % sodium dodecyl sulfate (SDS), 0.1 % lauroylsarcosine and 50 mg/ml salmon sperm DNA. The membrane was washed under stringent conditions and incubated in 0.1% CSPD solution (Boehringer Mannheim) as recommended by the manufacturer. Finally, the membrane was exposed to an X-ray film (Fuji, Japan).

DNA fragmentation assay. Se301 or CLS-79 cells (6.0×10^5 cells per 35-mm-diameter dish; Falcon 3801, Becton Dickinson) were inoculated with SeMNPV at a MOI of 5 PFU/cell, and harvested at 24 and 48 hrs p.i. Cellular DNA was isolated as described by Prikhod'ko and Miller (1996). The isolated DNA was resolved by electrophoresis in 1.2% agarose gel using TAE buffer (40 mmol/l Tris-acetate plus 2 mmol/l ethylenediamine tetraacetate (EDTA)). The gel was stained with ethidium bromide (0.5 µg/ml).

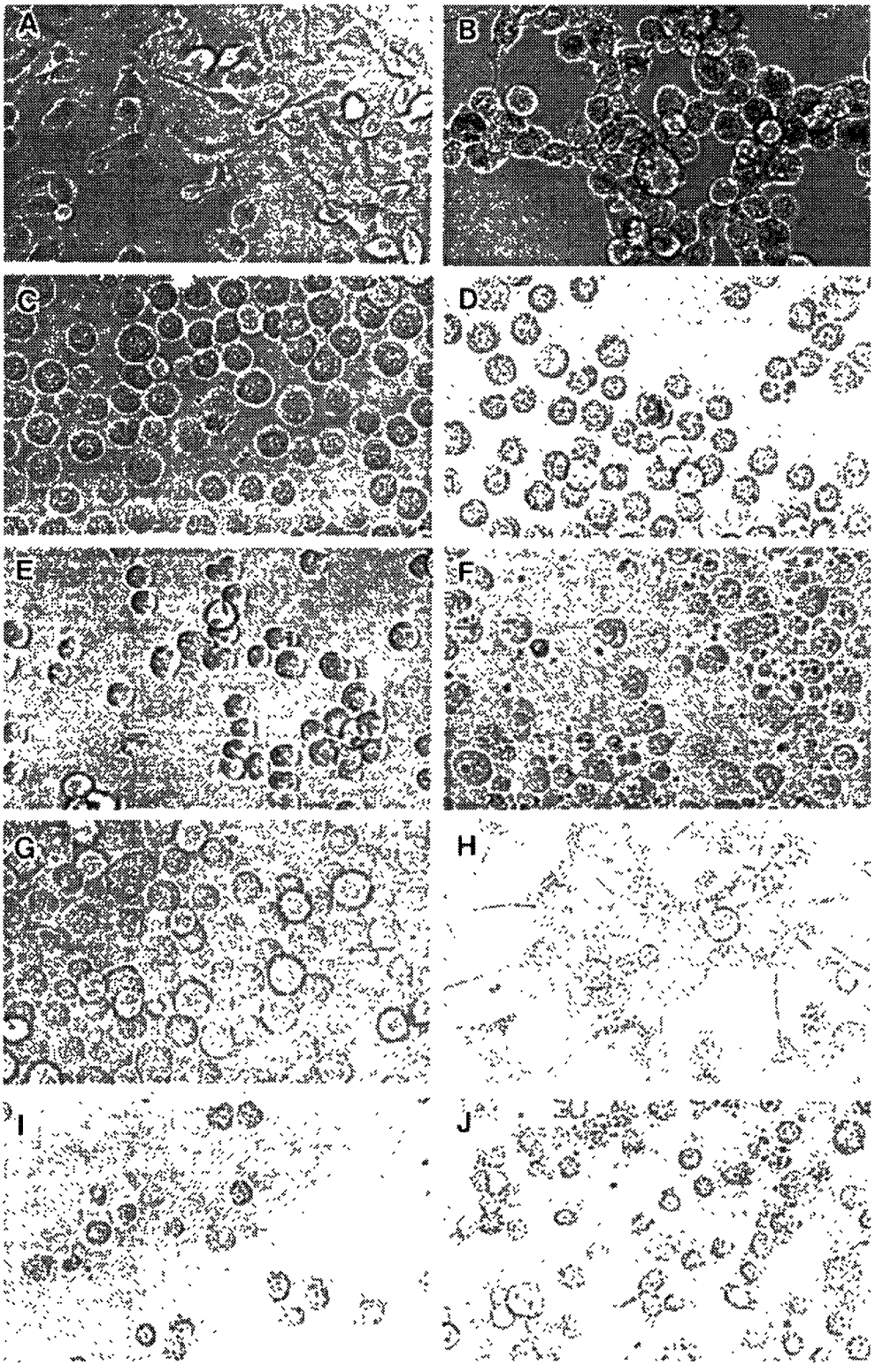

Fig. 1

Morphology of SeMNPV-infected and mock-infected lepidopteran cells at 72 hrs p.i.

Sc301 (A and B), SF21AEII (C and D), CLS-79 (E and F), hi-5 (G and H), and BmN4 (I and J) cells. Virus-infected cells (B, D, F, H and J). Mock-infected cells (A, C, E, G and I). Bar = 100 μm.

McIntosh AH, Grasela JJ (1994): Specificity of baculoviruses. In Maramorosch K, McIntosh AH (Eds): *Insect Cell Biotechnology.* CRC Press, Inc., Boca Raton, pp. 57–69.

Morris TD, Miller LK (1992): Promoter influence on baculovirus-mediated gene expression in permissive and non-permissive insect cell lines. *J. Virol.* **66**, 7397–7405.

Morris TD, Miller LK (1993): Characterization of productive and non-productive AcMNPV infection in selected insect cell lines. *Virology* **197**, 339–348.

Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Javis AW, Martelli GP, Mayo MA, Summers MD (1995): *Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses.* Springer Verlag, Wien-New York, pp. 104–113.

Palli SR, Caputo GF, Sohi SS, Brownwright AJ, Ladd TR, Cook BJ, Primavera M, Arif BM, Retnakaran A (1996): CfMNPV blocks AcMNPV-induced apoptosis in continuous midgut cell line. *Virology* **222**, 201–213.

Prikhod'ko EA, Miller LK (1996): Induction of apoptosis by baculovirus transactivator IE1. *J. Virol.* **70**, 7116–7124.

Smits PH, van de Vrie M, Vlak JM (1987): Nuclear polyhedrosis virus for control of *Spodoptera exigua* larvae on glasshouse crops. *Entomol. Exp. Appl.* **43**, 73–80.

Thiem SM (1997): Prospects for altering host range for baculovirus bioinsecticides. *Curr. Opin. Biotechnol.* **8**, 317–322.

Thiem SM, Du X, Quentin ME, Berner MM (1996): Identification of a baculovirus gene that promotes *Autographa californica* nuclear polyhedrosis virus replication in a non-permissive insect cell line. *J. Virol.* **70**, 2221–2229.

van Oers MM, Vlak JM (1997): The baculovirus 10-kDa protein. *J. Invertebr. Pathol.* **70**, 1–17.

Vlak JM (1993): Genetic engineering of baculoviruses for insect control. In Oakeshott J, Whitten MJ (Eds): *Molecular Approaches to Fundamental and Applied Entomology.* Springer Verlag, New York, pp. 90–127.

Vlak JM, van Frankenhuyzen K, Peters D, Gröner A (1981): Identification of a new nuclear polyhedrosis virus from *Spodoptera exigua. J. Invertebr. Pathol.* **38**, 297–298.

Yanase T, Yasunaga C, Hara T, Kawarabata T (1998): Co-infection of *Spodoptera exigua* and *Spodoptera frugiperda* cell lines with the nuclear polyhedrosis viruses of *Autographa californica* and *Spodoptera exigua*. *Intervirology* (in press).

Zanotto PMA, Kessing BD, Maruniak JE (1993): Phylogenetic interrelationships among baculoviruses: evolutionary rates and host associations. *J. Invertebr. Pathol.* **62**, 147–164.