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Characterization of baculovirus *Autographa californica* multiple nuclear polyhedrosis virus infection in mammalian cells

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Abstract

The baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) is used as a vector in many gene therapy studies. Wild-type AcMNPV infects many mammalian cell types in vitro, but does not replicate. We investigated the dynamics of AcMNPV genomic DNA in infected mammalian cells and used flow cytometric analysis to demonstrate that recombinant baculovirus containing a cytomegalovirus immediate early promoter/enhancer with green fluorescent protein (GFP) expressed high levels of GFP in Huh-7 cells, but not B16, Raw264.7, or YAC-1 cells. The addition of butyrate, a deacetylase inhibitor, markedly enhanced the percentage of GFP-expressing Huh-7 and B16 cells, but not Raw264.7 and YAC-1 cells. The addition of 5-aza-2′-deoxycytidine, a DNA methylation inhibitor, had no enhancing effect. Polymerase chain reaction analysis using AcMNPV-*gp64*-specific primers indicated that AcMNPV infected not only Huh-7 and B16 cells, but also Raw264.7 and YAC-1 cells in vitro. The genomic DNA was detected in Huh-7 and B16 cells 96 h after infection. Genomic AcMNPV DNA in YAC-1 cells was not transported to the nucleus. Luciferase assay indicated that AcMNPV *p35* gene mRNA and p35 promoter activity were clearly expressed only in Huh-7 and B16 cells. These results suggest that viral genomic DNA expression is restricted by different host cell factors, such as degradation, deacetylation, and inhibition of nuclear transport, depending on the mammalian cell type.

Keywords: Baculovirus; Vector; *gp64*; *p35*; Mammalian cells

The ability of baculovirus to infect insects has many applications. In particular, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) is used in many protein expression systems as a viral vector and plant insecticide.

AcMNPV has a double-stranded circular DNA genome of approximately 130 kbp containing more than 150 open-reading frames [1]. AcMNPV produced from infected cells has two viral phenotypes, occlusion-derived virus (ODV) and budded virus (BV) [2]. ODV enters midgut cells, and BV buds from infected midgut cells establish systemic infection by infecting hemocytes and other tissues in the larval lepidopteron host [3]. The viral genomic DNA of both phenotypes transports to the nucleus of the infected cells and replicates using nine essential viral genes, *ie-1*, *ie-2*, *p143*, *dnapol*, *lef-1*, *lef-2*, *lef-3*, *pe38*, and *p35* [4]. Moreover, the host range of AcMNPV in insect cells is detected by measuring the expression of viral helicase *p143* and pan-caspase inhibitor *p35* [5–8]. Argaud et al. [9] reported that recombinant AcMNPV *p143* replaced with *Bombyx mori* nuclear polyhedrosis virus (BmNPV) could replicate in non-permissive Bm5 cells. Clem and Miller [8], using a *p35* mutant virus, demonstrated protection of viral-induced apoptosis by expression of the AcMNPV *p35* gene within the host range.

AcMNPV infects a variety of mammalian cell types in vitro, but not certain hematopoietic cell lines, by unknown

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mechanisms. The inability of baculoviruses to replicate in mammalian cells makes them attractive candidate vectors for in vitro gene therapy studies [10,11]. These recombinant vectors contain compatible promoters and are highly effective in infecting primary hepatocyte and hepatoma cell lines, making them very useful tools for studies of hepatitis B and hepatitis C viruses [12–14].

To enhance gene delivery of the baculovirus vector into mammalian cells, a vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped baculovirus vector was generated to improve foreign gene expression in mammalian cells compared to wild-type baculovirus vector [15]. The VSV-G pseudotyped virus is thought to facilitate particle entry and escape from endolysosomes. Schauber et al. [16], however, reported that transduction of the baculovirus *gp64* pseudotyped lentiviral vector is similar to that of the VSV-G pseudotyped lentiviral vector, except for in hemapoietic cells. Furthermore, Tani et al. [17] suggested that baculovirus *gp64* vectors directly interact with general phospholipids in the surface membrane of mammalian cells, and the VSV-G pseudotyped virus interacts with general phosphatidylserine. Therefore, we hypothesized that baculovirus *gp64* mediates transduction in the presence of intracellular or viral factors.

In the present study, we investigated baculovirus infection and transduction in permissive and non-permissive mammalian cells in vitro. We report that AcMNPV penetrated not only Huh-7 cells, but also the hematopoietic cell lines, Raw264.7 and YAC-1 cells, while Raw264.7 and YAC-1 cells inhibited GFP gene expression. Nuclear transport of the AcMNPV genome was inhibited in YAC-1 cells. Our data suggest that AcMNPV might be useful for the development of more efficient baculovirus vectors for gene therapy.

Materials and methods

Animals and cell lines. Female C57BL/6 mice were purchased from Nippon SLC (Hamamatsu, Japan) and used at 6–8 weeks of age. A human hepatoma cell line (Huh-7), a mouse melanoma cell line (B16), a mouse lymphoma cell line (YAC-1), and a mouse macrophage cell line (Raw267.4) were obtained from the Riken Cell Bank (Wako, Japan). Huh-7, B16, and Raw264.7 cells were cultured in Dulbecco's modified Eagle's medium (Sigma Chemical Co., St Louis, MO) and YAC-1 in RPMI 1640 (Sigma Chemical Co.), both supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Where indicated, culture medium was supplemented with 5-aza-2′-deoxycytidine (Aza-C; Sigma Chemical Co.) or sodium butyrate (Wako Chemical Co., Osaka, Japan) after virus treatment.

Generation of recombinant baculovirus. Wild-type baculovirus AcMNPV was purchased from BD Bioscience (San Diego, CA). The GFP was moved from pLEGFP-C1 (BD Bioscience) to pcDNA3.1(+) (Invitrogen Corp., Carlsbad, CA) as a *Nhe*I-*Xho*I fragment to construct pcDNA.GFP. Viruses were constructed using shuttle vectors derived from pVL1393 (BD Bioscience). The shuttle plasmid DNA was digested using a *Sma*I-*Eco*RI fragment. A 3.1 kbp BamHI-MunI fragment from pcDNA.GFP, which contains the CMV-IE promoter/enhancer with a GFP gene and polyadenylation signal expression cassette, was inserted into the pVL1393 backbone (pVL1393/CMV-GFP). Recombinant baculovirus AcCMV-GFP was generated according to the manufacturer's instructions (BD Bioscience). Purification of the baculovirus was performed as described previously [18].

Transduction of mammalian cells by baculovirus. Cells were seeded in 35 mm culture dishes at 3×10^5 cells per dish. Culture medium was removed, replaced with virus treatment, and incubated for 1 h at 37 °C. After removal of the virus, fresh medium was added and cultures were incubated at 37 °C. Cultures were harvested, washed, and resuspended in phosphate-buffered saline. The GFP-expressing populations were analyzed by FACSCalibur (Becton Dickinson, Mountain View, CA).

Polymerase chain reaction. Total DNAs were extracted from cells using the GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma Chemical Co.) according to the manufacturer's instructions. Locations of the baculovirus genome were determined as described previously [15]. One-step polymerase chain reaction (PCR) was performed on 50 ng DNA samples using KOD-Plus—(Toyobo, Osaka, Japan). The specific primer pairs are presented in Table 1.

Construction of plasmids and luciferase assay. The enhancer *hr5* and p35 promoter were cloned from the AcMNPV genome to a pCR2.1-TOPO vector (Invitrogen), and the sequence homology was verified. The sequences of the specific primers were as follows: hr5: 5′-GAGCTCTTGC ACAATGTAACTAGTGCACTC-3′ (sense) and 5′-GCTAGCCGTCCG TTTGATTTAACG-3′ (anti-sense); p35 promoter: 5′-CTCGAGGTCGG TCACCATGTACAAAAG-3′ (sense) and 5′-AGATCTGCTCAAATGC TCACTTAATACAAG-3′ (anti-sense). The pGL.hr5.p35prom vector was constructed by a 705-bp SacI-NheI fragment containing hr5 and a 503-bp XhoI-BglII fragment containing the p35 promoter in a firefly luciferase reporter pGL3-basic vector (Promega Corp., Madison, WI). Transfections were performed by using Fugene 6 (Roche Applied Science), according to the manufacturer's instructions. Cells were plated at $0.8–3.0\times 10^5$ cells per well in 24-well plates 24 h before transfection. Reporter plasmid (400 ng) was mixed with 2 μl Fugene 6 in 50 μl serum-free medium, left for 15 min at room temperature, and then added to the cells. The pRL-TK vector (Promega) containing the herpes simplex virus thymidine kinase (HSV-TK) promoter driving the expression of a renilla luciferase reporter was used as an internal control for transfection efficiency (20 ng per transfection). Cells were harvested and lysed 24 h post-transfection. The firefly and renilla luciferase activities were measured using the Promega Dual luciferase assay system with 20 μl of cell extract according to the manufacturer's instructions with a luminometer (TD-20/20, Turner Designs, Sunnyvale, CA).

Results and discussion

Recombinant baculovirus-mediated GFP gene expression in mammalian cells in vitro

Infectivity and intensity of expression of AcCMV, which uses the CMV-IE promoter to express the GFP gene, was

Table 1
Primers used for PCR[a]

Gene	Sense	Anti-sense	Annealing temp (°C)
gp64	CTACTAGTAAATCAGTCACACC	CCAAGTTTTTAATCTTGTACGG	50
p35	GGTAGAAATCGACGTGTCCCAGA	CGTGAGCAAACGGCACAATAAC	56
p143	TAATGTATCCAGGGTCGGTGCTCT	CGCATCATCATGTCCAAAGTGGAC	56
G3PDH	TCCACCACCCTGTTGCTGTA	ACCACAGTCCATGCCATCAC	60

[a] All sequences are presented in the 5′–3′ direction.