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Short Communication

Expression of recombinant proteins by BEVS in a macula-like virus-free silkworm cell line

Masashi Iwanaga [a,*], Keita Tsukui [a], Koudai Uchiyama [a], Susumu Katsuma [b], Shigeo Imanishi [c,1], Hideki Kawasaki [a]

[a] Department of Agrobiology and Bioresources, Faculty of Agriculture, Utsunomiya University, Mine-machi 350, Utsunomiya-shi, Tochigi 321-8505, Japan
[b] Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan
[c] Genebank, National Institute of Agrobiological Science, Kannondai 2-1-2, Tsukuba-shi, Ibaraki 305-8602, Japan

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ABSTRACT

We previously established the first *Bombyx mori* macula-like virus (BmMLV)-free cell line (BmVF cells) from a *B. mori* embryo. In this study, we evaluated the expression of recombinant proteins in BmVF cells using a *B. mori* nucleopolyhedrovirus (BmNPV)-derived expression vector. Our results showed that BmVF cells are susceptible to BmNPV, and both the promoter activity of the *polyhedrin* gene and the post-translated modifications of a recombinant protein are equivalent between BmMLV-negative BmVF and -positive BmN4 cells. These findings indicate that persistent infection with BmMLV has no discernible effect on BmNPV-mediated protein production in *B. mori* cells.

1. Introduction

The *Bombyx mori* macula-like virus (BmMLV) is a member of the genus *Maculavirus*, family *Tymoviridae*, and contains a positive-strand RNA genome. Previously, we reported that a *B. mori* ovary-derived cell line, BmN4, was persistently infected with BmMLV (Katsuma et al., 2005). The replication strategy of BmMLV is largely unclear. Interestingly, almost all *B. mori*-derived cell lines have already been contaminated with BmMLV via an unknown infection route (Iwanaga et al., 2012). Recently, we established the first BmMLV-negative *B. mori* cell line, BmVF, derived from a *B. mori* embryo (Iwanaga et al., 2012).

B. mori nucleopolyhedrovirus (BmNPV) is a member of the group I *Alphabaculovirus* and is used for the Baculovirus Expression Vector System (BEVS). Since many of the post-translational modifications present in the eukaryote are performed in baculovirus-infected cells, the BmNPV-based BEVS is frequently used for the expression of recombinant proteins that are biologically active and functional as well as for a native protein (reviewed in Rohrmann (2008)). For the BmNPV-based BEVS, *B. mori*-derived cell lines that are susceptible to BmNPV have become more important tools for the generation and/or propagation of recombinant NPVs. Therefore, the contamination of *B. mori* cell lines with BmMLV will cause a serious problem in safety aspects of the production of recombinant proteins. However, it is still unclear whether BmMLV-free BmVF cells are available in the BmNPV-based BEVS.

In this study, to assess the effect of BmMLV infection on production of recombinant proteins by the BEVS, properties of recombinant proteins expressed in BmMLV-negative BmVF and -positive BmN4 cells were compared. In addition, we attempted to culture the BmVF cells in a serum-free medium (SFM).

2. Materials and methods

2.1. Cells, virus, and plaque assay

BmVF and BmN4 cell lines were maintained as described previously on IPL-41 and TC-100 media supplemented with 10% fetal bovine serum (FBS), respectively (Iwanaga et al., 2004, 2012). To generate BmMLV-free BmNPV, viral genome DNA was extracted from occlusion bodies (OBs) or budded viruses (BVs) of BmNPV T3 (Maeda et al., 1985) and introduced into BmVF cells by transfection (Iwanaga et al., 2012). At 120 h posttransfection, a culture medium containing BVs was collected and subjected to a plaque

* Corresponding author. Fax: +81 28 649 5401.
E-mail address: iwanaga@cc.utsunomiya-u.ac.jp (M. Iwanaga).
[1] Present address: PrevenTec, Inc., Owashi 1-2, Tsukuba-shi, Ibaraki 305-0851, Japan.

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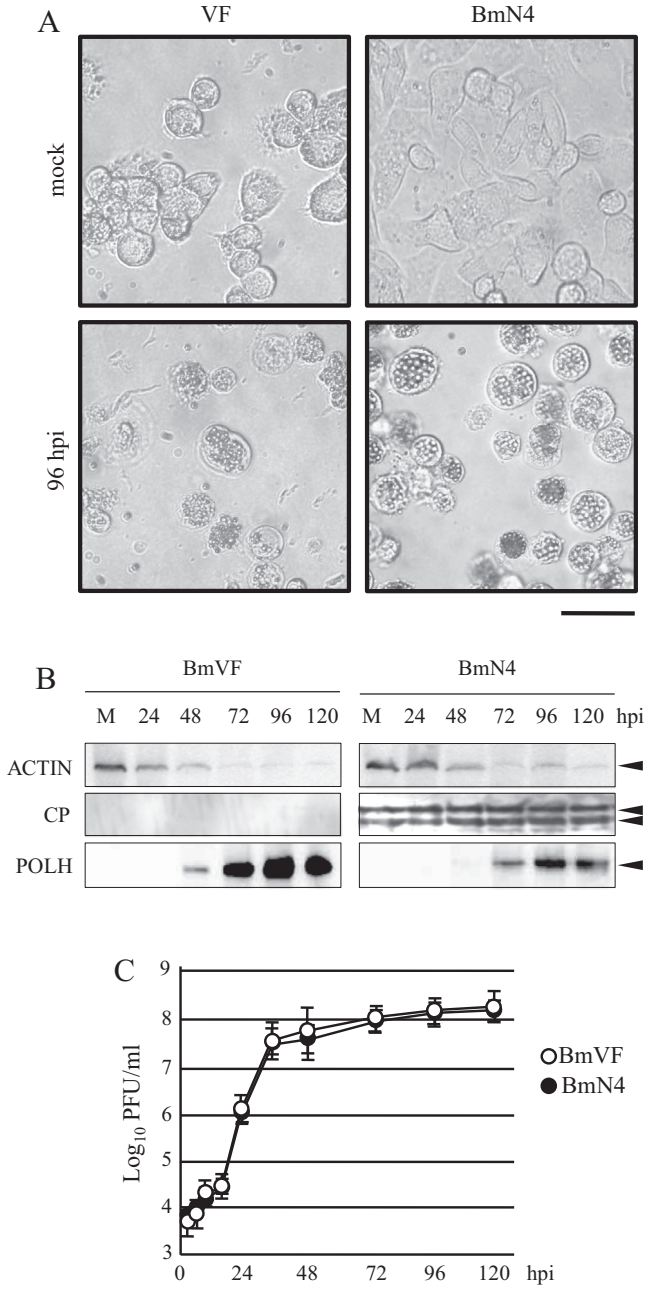

Fig. 1. Susceptibility of BmVF cells to BmNPV. (A) The morphology of BmMLV-negative BmVF and BmMLV-positive BmN4 cells at 84 h post-BmNPV-infection. The scale bar is 50 μm. (B) Expression of *B. mori*, BmMLV, and BmNPV proteins. BmVF and BmN4 cells were inoculated with BmNPV. Extracts were prepared from 1×10^5 mock- or BmNPV-infected cells harvested at the indicated times postinfection and were subjected to SDS–PAGE followed by Western blotting. Arrowheads indicate BmACTIN (ACTIN), BmMLV CP (CP), and BmNPV POLYHEDRIN (POLH). (C) Growth curves of BmNPV in *B. mori* cell lines. BmVF (open circles) and BmN4 (closed circles) cells were inoculated with BmNPV at an MOI of 10. At 2, 6, 12, 18, 24, 36, 48, 72, 96, and 120 hpi, a culture supernatant was collected and subjected to a plaque assay on BmN cells. The results represent the average of three independent experiments; standard errors are indicated.

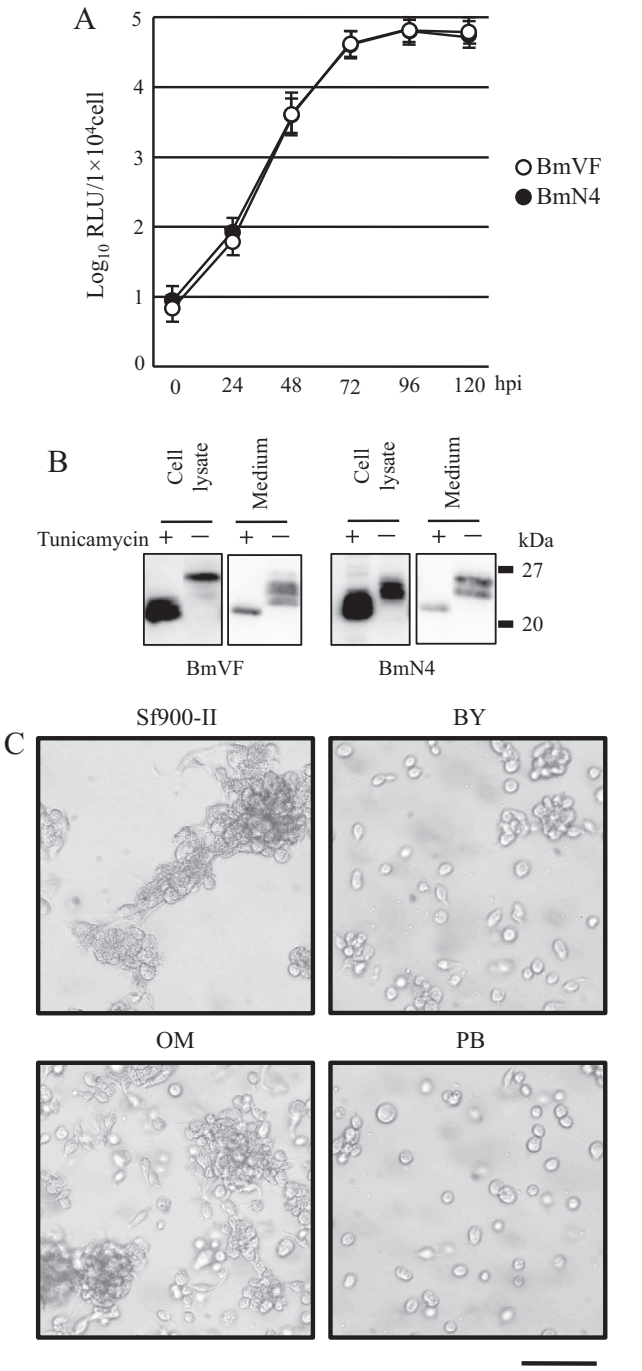

Fig. 2. Expression of recombinant proteins by BmNPV-based BEVS. (A) *Polyhedrin* promoter activity in *B. mori* cell lines. BmVF (open circle) and BmN4 (closed circle) cells were infected with Luc-Bm, which expresses the *luciferase* gene under the control of the *polyhedrin* promoter. At the indicated time postinfection, virus-infected cells were harvested and subjected to a luciferase assay. The results represent the average of three independent experiments; standard errors are indicated. (B) Expression of mIL-3 in *B. mori* cell lines. BmVF and BmN4 cells were inoculated with IL-Bm, which expresses the *mIL-3* gene under the control of the *polyhedrin* promoter. After inoculation, the cells were treated with tunicamycin (10 μg/ml) or DMSO and harvested for 72 h. The extracts, prepared from 1×10^5 BmNPV-infected cells, and the culture media were collected and subjected to SDS–PAGE followed by Western blotting using an anti-FLAG antibody. Size markers are indicated on the right side of the panel. (C) Adaptation of BmVF cells to the SFM. The morphology of BmVF cells after 10 passages using four commercial insect serum-free media, SF900-II, BY, OM, and PB. The scale bar is 50 μm.

assay on BmN4 cells as appropriate to determine viral titers (Iwanaga et al., 2004). The cells were infected with BmMLV-free BmNPV at a multiplicity of infection (MOI) of 10. The BmMLV-free recombinant BmNPVs expressing a firefly luciferase or mouse interleukin-3 (mIL-3) under the control of a *polyhedrin* promoter, Luc-Bm or IL-Bm (Nakanishi et al., 2010), were generated as described above. Statistical analysis was carried out using an unpaired *t*-test with KaleidaGraph computer software version 4.1 (Synergy Software, Reading, PA, USA).