Virology 498 (2016) 240–249

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Bombyx mori nucleopolyhedrovirus BM5 protein regulates progeny virus production and viral gene expression

Ryuhei Kokusho*, Yoshikazu Koh, Masaru Fujimoto, Toru Shimada, Susumu Katsuma*

Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:
Received 7 April 2016
Returned to author for revisions
27 August 2016
Accepted 30 August 2016
Available online 8 September 2016

Keywords:
Baculovirus
BmNPV
Bm5
DUF3627
Progeny production
Viral gene expression
Subcellular localization
BmN cells
Bombyx mori larvae

ABSTRACT

Bombyx mori nucleopolyhedrovirus (BmNPV) *orf5* (*Bm5*) is a core gene of lepidopteran baculoviruses and encodes the protein with the conserved amino acid residues (DUF3627) in its C-terminus. Here, we found that *Bm5* disruption resulted in lower titers of budded viruses and fewer numbers of occlusion bodies (OBs) in *B. mori* cultured cells and larvae, although viral genome replication was not affected. *Bm5* disruption also caused aberrant expression of various viral genes at the very late stage of infection. Immunocytochemical analysis revealed that BM5 localized to the nuclear membrane. We also found that DUF3627 is important for OB production, transcriptional regulation of viral genes, and subcellular localization of BM5. Compared with wild-type BmNPV infection, larval death was delayed when *B. mori* larvae were infected with *Bm5* mutants. These results suggest that BM5 is involved in progeny virus production and regulation of viral gene expression at the very late stage of infection.

1. Introduction

The family *Baculoviridae* encompasses a large group of insect viruses that infect lepidopteran, hymenopteran, and dipteran insects. Baculoviruses have a large (80–180 kb), circular, supercoiled, and double-stranded DNA genome, which generally encodes >100 protein-coding genes (Rohrmann, 2013). During their infection cycle, baculoviruses produce two types of virions to replicate efficiently within the host body and spread their progeny among insects. Budded virions (BVs) are required for cell-to-cell infection and are involved in the spread of the virus within an infected host. Occlusion-derived viruses (ODVs) are enclosed within an occlusion body (OB) that protects and transmits ODVs from insect to insect via oral infection (Granados and Lawler, 1981; Keddie et al., 1989). At the late stage of infection, infected larvae show enhanced locomotory activity; they vigorously move about and finally climb to the upper plant foliage (Goulson, 1997; Kamita et al., 2005; Hoover et al., 2011), where their cadavers are degraded by viral proteinase (V-CATH) and chitinase (Ohkawa et al., 1994; Hawtin et al., 1997).

Bombyx mori nucleopolyhedrovirus (BmNPV) *orf5* (*Bm5*), a homolog of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *Ac13*, is a viral late gene encoding a protein (BM5) of 331 amino acids. Previous studies reported that BM5 and its homologs were not components of BV or ODV (Zhou et al., 2010; Hou et al., 2013; Braconi et al., 2014), and BM5 is a nonstructural protein primarily localized to the cytoplasm (Zhou et al., 2010). Although *Bm5* is one of the 62 core genes of lepidopteran baculoviruses (Jehle et al., 2006), its biological role remains unknown. BM5 possesses a protein region of unknown function, designated as DUF3627, at amino acid residues from +223 to +308. DUF3627 is one of the homologous protein sequences annotated as "Domains of Unknown Functions" (DUFs) in the Pfam database (Finn et al., 2014). Because DUFs including DUF3627 were provided only on the basis of domain sequence similarity, represented by multiple sequence alignments and hidden Markov models, their structures and functions are not experimentally characterized (Bateman et al., 2010). At present, nucleotide sequences of 330 genes containing the DUF3627 region have been deposited in a public database (NCBI, http://www.ncbi.nlm.nih.gov/), 162 of which are encoded in the genome of baculoviruses and 163 in that of nucleocytoplasmic large DNA viruses including poxviruses, iridoviruses, ascoviruses and mimiviruses, implying the importance of DUF3627 for wide range of large DNA viruses. In baculoviruses, DUF3627 is conserved in BM5 homologs of all lepidopteran nucleopolyhedroviruses but not in

* Corresponding authors.
E-mail addresses: kokusho@ss.ab.a.u-tokyo.ac.jp (R. Kokusho), katsuma@ss.ab.a.u-tokyo.ac.jp (S. Katsuma).

http://dx.doi.org/10.1016/j.virol.2016.08.032
0042-6822/

those of lepidopteran granuloviruses. In addition, some types of the *baculovirus repeated orf* (*bro*) gene products also possess DUF3627. For example, BmNPV has three such BROs; BRO-A, BRO-C, and BRO-D. *bro-d* is an essential gene and at least one of *bro-a* or *bro-c* is required for virus propagation (Kang et al., 1999), suggesting that these BRO proteins containing DUF3627 serve an important role in viral infection. However, the detailed function of DUF3627 has not been yet characterized.

Here, we report the characterization of *Bm5*-disrupted BmNPVs in *B. mori* cultured cells and in *B. mori* larvae. We also examined the role of the DUF3627 region of the BM5 protein during BmNPV infection.

2. Results

2.1. OB and BV production of Bm5 mutants in cultured cells

To reveal the function of the BM5 protein and its DUF3627 region, four mutant BmNPVs were generated (Fig. 1). Bm5D and Bm5stop are *Bm5*-knockout mutants whose *Bm5* gene was disrupted by introducing an *hsp70-lacZ* cassette or a stop codon, respectively. Bm5DUFD is a mutant virus lacking only the DUF3627 region of BM5. Bm5DR is a revertant virus of Bm5D. Using these *Bm5* mutants, we first examined OB and BV production in BmN cells at 72 h postinfection (hpi). An inspection of cells by light

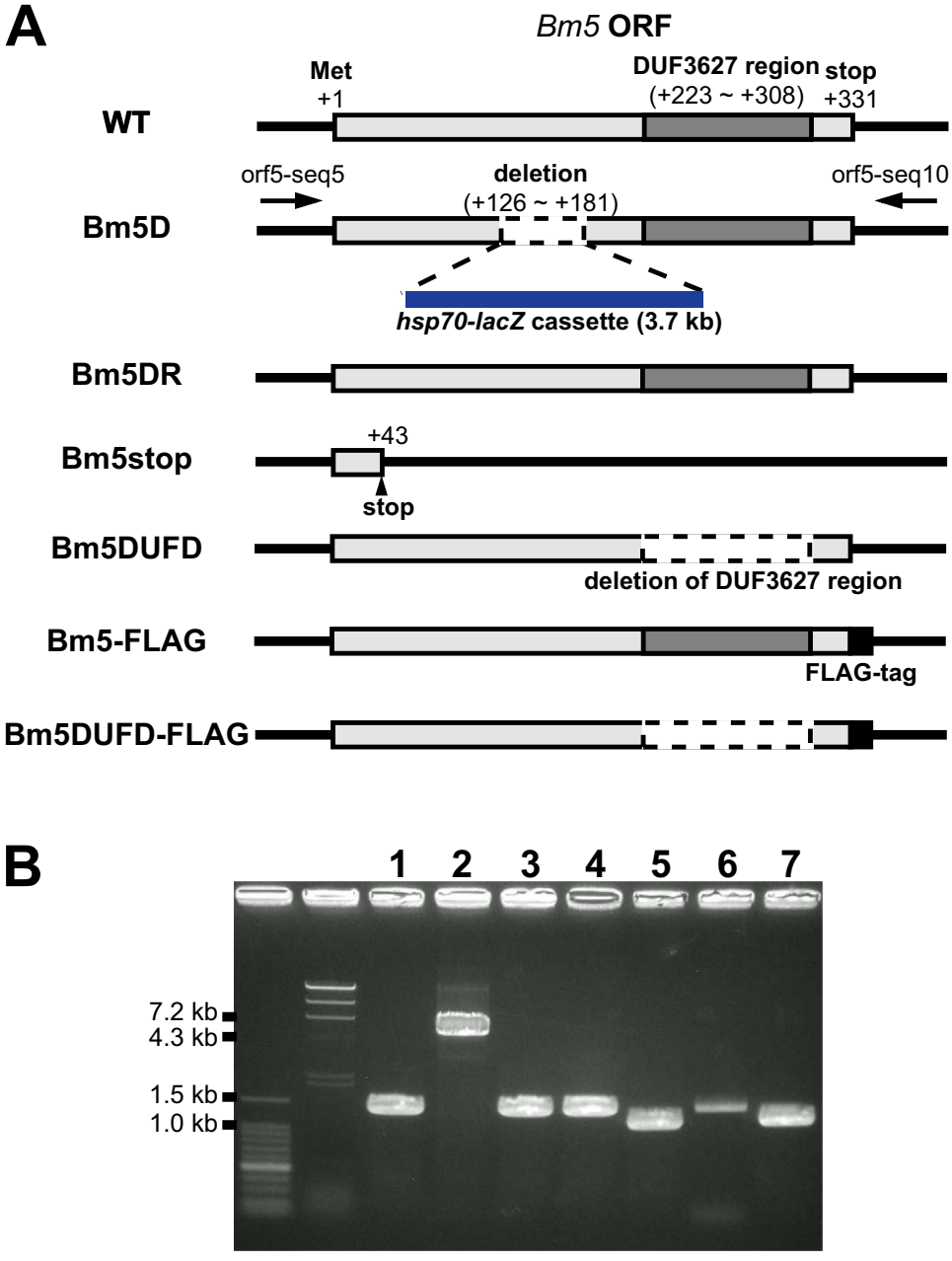

Fig. 1. *Construction of Bm5-mutated BmNPVs.* (A) Schematic representation of *Bm5* mutant BmNPVs. In Bm5D, amino acid residues from +126 to +181 were replaced by the *hsp70-lacZ* cassette. Bm5DR is a revertant of Bm5D. Bm5stop has an additional stop codon by the single base substitution at the amino acid residue +43. Bm5DUFD lacks amino acids from +223 to +308 that correspond to the DUF3627 region. Bm5-FLAG and Bm5DUFD-FLAG are C-terminal FLAG-tagged variants of Bm5DR and Bm5DUFD, respectively. All the diagrams except the *hsp70-lacZ* cassette keep actual magnification ratio. Scheme for construction of mutant BmNPVs is described in detail in Materials and methods. (B) PCR analysis of the genome of WT (lane 1), Bm5D (lane 2), Bm5DR (lane 3), Bm5stop (lane 4), Bm5DUFD (lane 5), Bm5-FLAG (lane 6), and Bm5DUFD-FLAG (lane 7). Deletion of *Bm5* or the DUF3627 region was confirmed by PCR using the primers orf5-seq5 and orf5-seq10. Expected band sizes are 1380 bp (lane 1, 3, and 4), 5.0 kb (lane 2), 1122 bp (lane 5), 1404 bp (lane 6), and 1146 bp (lane 7), respectively. The left lanes contain size markers.