Virus Research 233 (2017) 70–76

Contents lists available at ScienceDirect

Virus Research

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

P143 proteins from heterologous nucleopolyhedroviruses induce apoptosis in BM-N cells derived from the silkworm *Bombyx mori*

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ARTICLE INFO

Article history:
Received 15 February 2017
Received in revised form 9 March 2017
Accepted 10 March 2017
Available online 16 March 2017

Keywords:
Nucleopolyhedrovirus
P143 protein
Bombyx mori
Apoptosis
BmNPV
AcMNPV

ABSTRACT

We previously demonstrated that ribosomal RNA (rRNA) of *Bombyx mori* BM-N cells is rapidly degraded upon infection with heterologous nucleopolyhedroviruses (NPVs), including *Autographa californica* multiple NPV (AcMNPV), *Hyphantria cunea* MNPV, *Spodoptera exigua* MNPV and *S. litura* MNPV, and that this response is triggered by viral P143 proteins. The transient expression of P143 proteins from heterologous NPVs was also shown to induce apoptosis and caspase-3-like protease activation in BM-N cells. In the present study, we conducted a transient expression assay using BM-N cells expressing mutant AcMNPV P143 (Ac-P143) proteins and demonstrated that five amino acid residues cooperatively participate in Ac-P143 protein-triggered apoptosis of BM-N cells. Notably, these five residues were previously shown to be required for triggering rRNA degradation in BM-N cells. As rRNA degradation in BM-N cells does not result from apoptosis, the present results suggest that Ac-P143-triggered rRNA degradation is the upstream signal for apoptosis induction in BM-N cells. We further showed that P143 protein-triggered apoptosis does not occur in *S. frugiperda* Sf9 or *Lymantria dispar* Ld652Y cells, indicating that apoptosis induction by heterologous P143 proteins is a BM-N cell-specific response. In addition, the observed induction of apoptosis in BM-N cells was found to be mediated by activation of the initiator caspase Bm-Dronc. Taken together, these results suggest that BM-N cells evolved a unique antiviral system that recognizes heterologous NPV P143 proteins to induce rRNA degradation and caspase-dependent apoptosis.

1. Introduction

The baculovirus *p143* gene is a core gene that is conserved in all baculovirus genomes sequenced to date (Carstens, 2009; van Oers and Vlak, 2007) and encodes a DNA helicase that is essential for viral DNA replication (Lu and Carstens, 1991, 1993; McDougal and Guarino, 2000, 2001). In *Spodoptera frugiperda* Sf21 cells infected with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), P143 protein is synthesized early in the infection process and is transported into the nucleus by virus-encoded LEF-3 (late expression factor-3) protein (Wu and Carstens, 1998). Upon entering the nucleus, P143 protein forms a viral DNA replication complex and localizes in the virogenic stroma, which is the site of virus DNA replication and nucleocapsid morphogenesis (Nagamine et al., 2006, 2008). Consistent with its observed DNA helicase activity, P143 protein binds both single- and double-stranded DNA (Laufs et al., 1997; McDougal and Guarino, 2000, 2001). P143 proteins also function in a virus species-specific manner with respect to viral DNA replication (Ahrens and Rohrmann, 1996; Bideshi and Federici, 2000; Chen et al., 2004; Heldens et al., 1997). Recently, it was found that AcMNPV *p143* gene contains a unique sequence that serves as an origin of DNA replication in both insect and mammalian cells (Wu et al., 2014).

P143 proteins serve as host range determinants of AcMNPV and *Bombyx mori* NPV (BmNPV) in *B. mori* cells. BmNPV productively infects cell lines derived from *B. mori*, but does not replicate in Sf9 or Sf21 cells derived from *S. frugiperda* (Katou et al., 2001, 2006), whereas AcMNPV is unable to replicate in *B. mori* cells, but productively infects Sf9 or Sf21 cells. Although AcMNPV and BmNPV have distinct host range properties, these baculoviruses are closely related with respect to genome organization and content, including gene structure, and share over 90% nucleotide sequence identity in approximately 75% of all genomic regions (Ayres et al., 1994; Cohen et al., 2009; Gomi et al., 1999). A notable feature of AcMNPV infection of *B. mori* cells is the induction of global protein synthesis shutdown, in which both cellular and viral protein translation is arrested (Kamita and Maeda, 1993). Global protein synthesis shutdown in *B. mori* cells is induced by the product of the AcMNPV *p143*

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http://dx.doi.org/10.1016/j.virusres.2017.03.012
0168-1702/

gene (Argaud et al., 1998; Croizier et al., 1994; Ikeda et al., 2013, 2015; Kamita and Maeda, 1993, 1997; Maeda et al., 1993).

We previously reported that *B. mori* ribosomal RNA (rRNA) undergoes rapid and extensive degradation upon infection of BM-N cells with heterologous NPVs, including AcMNPV, *Hyphantria cunea* MNPV (HycuMNPV), *S. exigua* MNPV (SeMNPV) and *S. litura* MNPV (SpltMNPV) (Hamajima et al., 2013). Gene screening with a cosmid library of the HycuMNPV genome identified *p143* as the gene responsible for the rRNA degradation, and transient expression of heterologous NPV P143 proteins in BM-N cells triggered rRNA degradation (Hamajima et al., 2013). Mutational analyses further demonstrated that six to eight amino acid residues of Ac-P143 protein participate in inducing rRNA degradation and restricting NPV replication in BM-N cells. These findings suggest that rRNA degradation is the primary cause for restricted viral replication in *B. mori* cells (Hamajima et al., 2014, 2015). In addition to the induction of rRNA degradation, BM-N cells transiently expressing heterologous P143 proteins induce caspase-dependent apoptosis (Hamajima et al., 2013).

In the present study, we demonstrated that P143-triggered apoptosis is mediated by activation of the *B. mori* homolog of the initiator caspase Dronc (Bm-Dronc), and that the apoptosis is specifically induced in BM-N cells. Five amino acid residues of Ac-P143 protein were shown to cooperatively participate in apoptosis induction in BM-N cells. The identified residues are also responsible for rRNA degradation (Hamajima et al., 2015), suggesting that P143-triggered rRNA degradation leads to the apoptosis of BM-N cells.

2. Materials and methods

2.1. Cells

BM-N cells from the silkworm *Bombyx mori* (Volkman and Goldsmith, 1982), Sf9 cells from the fall armyworm *Spodoptera frugiperda* (Summers and Smith, 1987) and IPLB-Ld652Y (Ld652Y) cells from the gypsy moth *Lymantria dispar* (Goodwin et al., 1978) were cultured at 28 °C in TC100 medium (AppliChem) supplemented with 0.26% tryptose broth (Sigma) and 10% fetal bovine serum, as described previously (Ikeda et al., 2011).

2.2. Construction of expression plasmids

To construct the expression plasmids pIE1-2/HycuP143Egfp, pIE1-2/SeP143Egfp and pIE1-2/SpltP143Egfp for EGFP-tagged Hycu-P143, Se-P143 and Splt-P143 proteins, respectively, pIE1-2/Egfp (Ikeda et al., 2004) was linearized by inverse-PCR using the primers GFPfusionC-S and GFPfusionC-AS (Table S1). The coding regions of *hycu-p143*, *se-p143* and *splt-p143* were PCR-amplified from pPIGA3hr5/Hycu-p143, pPIGA3hr5/Se-p143 and pPIGA3hr5/Splt-p143 (Hamajima et al., 2013), respectively, using the respective primer pairs (Table S1), which contained 15-nt extensions complementary to the ends of linearized pIE1-2/Egfp. The amplified coding regions of the respective genes were cloned into linearized pIE1-2/Egfp using an In-Fusion HD Cloning Kit (Clontech), yielding pIE1-2/HycuP143Egfp, pIE1-2/SeP143Egfp and pIE1-2/SpltP143Egfp. The expression plasmids pIE1-2/AcP143Egfp and pIE1-2/BmP143Egfp for EGFP-tagged Ac-P143 and EGFP-tagged Bm-P143 proteins, respectively, and the plasmids expressing EGFP-tagged mutant Ac-P143 and Bm-P143 proteins (see Fig. 5A, a–l) were generated previously (Hamajima et al., 2015). All plasmid sequences were confirmed by nucleotide sequencing using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit and ABI 3130 Genetic Analyzer (Applied Biosystems), as described previously (Suganuma et al., 2011).

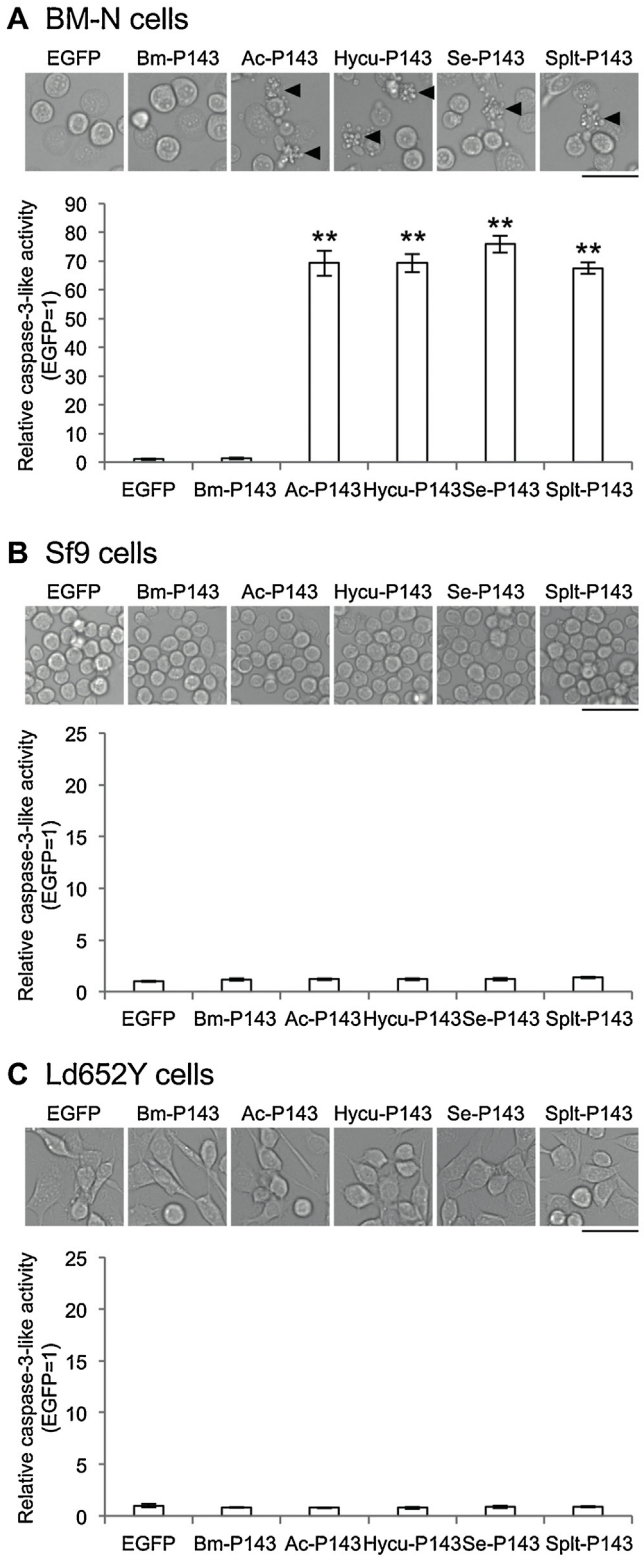

Fig. 1. P143 proteins of heterologous NPVs trigger apoptosis in BM-N cells, but not in Sf9 or Ld652Y cells. BM-N (A), Sf9 (B) and Ld652Y cells (C) were transfected with 2 μg of pIE1-2/Egfp (EGFP), pIE1-2/BmP143Egfp (Bm-P143), pIE1-2/AcP143Egfp (Ac-P143), pIE1-2/HycuP143Egfp (Hycu-P143), pIE1-2/SeP143Egfp (Se-P143) or pIE1-2/SpltP143Egfp (Splt-P143). At 72 h post-transfection, the cells were examined for apoptosis (upper panels) and caspase-3-like protease activity (lower panels). The arrowheads show apoptotic cells. The scales bars, 50 μm. Caspase-3-like protease activities were normalized to that of EGFP-transfected cells. The error bars in graphs indicate the standard deviations of the means from three determinations. Data were analyzed by Dunnett's test using EGFP-expressing cells as the competitor. The asterisks (**) indicate statistically significant differences ($p < 0.01$).