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Identification of amino acid residues of AcMNPV P143 protein involved in rRNA degradation and restricted viral replication in BM-N cells from the silkworm *Bombyx mori*

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ABSTRACT

We previously demonstrated that rRNA undergoes rapid and extensive degradation in *Bombyx mori* BM-N cells upon infection with AcMNPV, which is triggered by AcMNPV P143 (Ac-P143) protein. Here, we showed that six amino acid residues of Ac-P143 protein, distributing between positions 514 and 599, are involved in rRNA degradation in BM-N cells. The six residues are highly conserved among P143 proteins from AcMNPV, HycuMNPV, SeMNPV and SpltMNPV, which trigger rRNA degradation in BM-N cells upon infection, but are only partially conserved in Bm-P143 protein, which does not induce rRNA degradation in BM-N cells. We also demonstrated that substitution of only two selected residues (N565S/L578F) of Bm-P143 protein with the corresponding Ac-P143 protein residues generates a mutant Bm-P143 protein that is capable of triggering rRNA degradation in BM-N cells. These results indicate that BmNPV evolved a unique P143 protein to evade the antiviral response and allow replication in *B. mori* cells.

Introduction

Nucleopolyhedroviruses (NPVs) are large, enveloped, insect-pathogenic viruses that are members of the family *Baculoviridae* and contain a double-stranded, closed-circular DNA genome of approximately 80–180 kbp (Herniou et al., 2012). The majority of NPVs display a narrow host range that is generally limited to close relatives of the insect(s) from which the respective NPVs were originally isolated. A number of baculovirus genes, including *hrf-1*, *hcf-1*, *lef7*, *p35*, *iap*, *p143* and *hycu-ep32*, have distinct functional roles during infection and also contribute to host range determination (Miller and Lu, 1997; Shirata et al., 2004, 2010; Thiem, 1997; Thiem and Cheng, 2009), suggesting that baculovirus host range is determined by diverse underlying mechanisms.

Autographa californica multiple NPV (AcMNPV) and *Bombyx mori* NPV (BmNPV) are closely related with respect to genome organization, gene content and nucleotide sequences of conserved genes, with the corresponding ORFs showing approximately 90% overall amino acid sequence identity between the two viral genomes (Ayres et al., 1994; Cohen et al., 2009; Gomi et al., 1999). Despite such a high degree of relatedness, these viruses display a striking difference in host range specificity. Compared to BmNPV, which is restricted to replication in cell lines from the silkworm, *B. mori*, and its wild ancestor, the wild silkworm, *Bombyx mandarina* (Iwanaga et al., 2009; Shirata et al., 1999), AcMNPV exhibits exceptionally broad host range and is able to infect a minimum of 32 lepidopteran insect species within 12 families (Granados and Williams, 1986). However, AcMNPV is unable to replicate productively in *B. mori* cells (Grasela et al., 2000; Kamita and Maeda, 1993; Morris and Miller, 1993; Shirata et al., 1999), and AcMNPV infection of *B. mori* cells induces global protein synthesis shutdown, in which both viral and cellular protein syntheses are completely arrested (Kamita and Maeda, 1993).

Previous studies have demonstrated that a recombinant AcMNPV harboring a specific DNA fragment of the BmNPV *p143* gene (*bm-p143*) in place of the corresponding AcMNPV *p143* gene (*ac-p143*) sequence no longer triggers global protein synthesis shutdown and is able to replicate successfully in *B. mori* cells. This finding suggests that P143 proteins encoded in the genomes of AcMNPV and BmNPV serve as host range determinants for the infection of *B. mori* cells (Croizier et al., 1994; Ikeda et al., 2013, 2015; Kamita and Maeda, 1993; Maeda et al., 1993). Mutational studies have demonstrated that the substitution of even a single amino acid residue of Ac-P143 with the corresponding residue of Bm-P143 is sufficient to enable AcMNPV to replicate in *B. mori* cells and larvae (Kamita and Maeda, 1997). However, the

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molecular mechanism underlying the restriction of AcMNPV replication in *B. mori* cells by Ac-P143 has not been determined.

Upon infection of *B. mori* cells with AcMNPV, cellular rRNA is rapidly and extensively degraded (Fujita et al., 2005; Hamajima et al., 2013), suggesting that the global protein synthesis shutdown observed in AcMNPV-infected *B. mori* cells is associated with rRNA degradation. The results of genetic screening and transient expression analyses indicated that Ac-P143 is involved in AcMNPV-triggered rRNA degradation (Hamajima et al., 2013). In the present study, we identified six amino acid residues of Ac-P143 that are involved in Ac-P143-triggered rRNA degradation in BM-N cells. Further, we found that the six amino acid residues of Ac-P143 protein are also involved in restricted viral replication in BM-N cells, providing supporting evidence that rRNA degradation is the primary cause for global protein synthesis shutdown and restricted viral replication in AcMNPV-infected *B. mori* cells. Comparative amino acid sequence alignments of P143 proteins revealed that the six identified amino acid residues from AcMNPV and the other NPVs that also trigger rRNA degradation in *B. mori* cells markedly differ from the corresponding amino acids in Bm-P143 protein, suggesting that BmNPV evolved a unique P143 protein to evade the antiviral response mechanism of *B. mori* cells against NPV infection.

Results

ScH sequence of Ac-P143 protein is involved in rRNA degradation and restricted viral replication in BM-N cells

A 572-bp *Sac*I-*Hin*dIII (ScH) sequence within the *ac-p143* (AcScH) gene encoded in the AcMNPV genome (Fig. 1) was previously shown to be involved in restricted viral replication in *B. mori* BmN cells, triggering global protein synthesis shutdown (Maeda et al., 1993). In addition, it was shown that AcMNPV containing the ScH sequence from BmNPV P143 (BmScH) in place of AcScH is able to replicate productively in BmN cells (Maeda et al., 1993). Here, to determine whether the AcScH sequence is also involved in AcMNPV-triggered rRNA degradation in BM-N cells, the plasmids pIE1-2/Acp143Egfp, pIE1-2/Bmp143Egfp and pIE1-2/Acp143(BmScH)Egfp (Fig. 2A), which express EGFP-tagged wild-type (wt) Ac-P143, Bm-P143 and recombinant Ac-P143 protein containing the BmScH sequence in place of the AcScH sequence (Ac-P143(BmScH)), respectively, were constructed and transfected into BM-N cells. At 0 and 72 h post-transfection, total RNAs were isolated and analyzed using the MultiNA microchip electrophoresis system. The analysis confirmed that Ac-P143 protein triggered rRNA degradation in BM-N cells, as evidenced by the formation of a weak, but clearly detectable, band composed of ~1400 nt rRNA fragments (Fig. 2B; Hamajima et al., 2013). In contrast, no detectable ~1400 nt rRNA fragments were observed in BM-N cells expressing Ac-P143(BmScH) or Bm-P143 proteins (Fig. 2B). These results are consistent with previous results

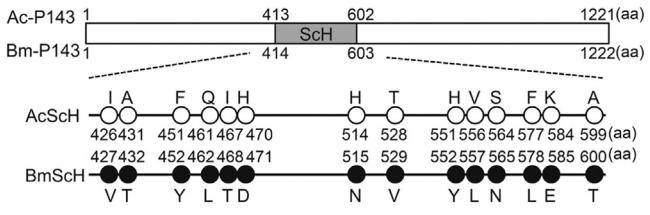

Fig. 1. Schematic representation of P143 proteins of AcMNPV (Ac-P143) and BmNPV (Bm-P143), showing amino acid sequences of the ScH regions (a 572-bp *Sac*I-*Hin*dIII DNA fragment of the *ac-p143* gene; upper panel) and the 14 residues that differ between the Ac-P143 ScH (AcScH) and Bm-P143 ScH (BmScH) amino acid sequences (lower panel).

(Hamajima et al., 2013) and indicated that the AcScH sequence is also involved in AcMNPV-triggered rRNA degradation in BM-N cells.

To determine if rRNA degradation is involved in global protein synthesis shutdown and restricted viral replication in BM-N cells expressing Ac-P143, single-cell based analysis of BM-N cells transfected with plasmids expressing EGFP-tagged Ac-P143(BmScH), Ac-P143 and Bm-P143 proteins was performed (Fig. 2C). At 48 h post-transfection, the cells were infected with BmNPV for 48 h and then examined for polyhedra production and expression of wt and mutant P143 proteins displaying EGFP fluorescence. In cell cultures transfected with pIE1-2/Acp143(BmScH)Egfp and pIE1-2/Bmp143Egfp, polyhedra were detected in BM-N cells without detectable fluorescence and in cells expressing mutant Ac-P143(BmScH) and wt Bm-P143 proteins (Fig. 2C). Quantification of polyhedra-producing cells revealed that approximately 20% of BM-N cells expressing AcP143(BmScH) and Bm-P143 proteins produced polyhedra (Fig. 2D). In contrast, in the pIE1-2/Acp143Egfp-transfected culture, polyhedra were produced exclusively in BM-N cells not expressing Ac-P143 protein (Fig. 2C), as no Ac-P143 protein-expressing cells produced polyhedra (Fig. 2D). These results indicated that the AcScH sequence of Ac-P143 protein is involved in both rRNA degradation and restricted viral replication in BM-N cells.

Mutant Ac-P143 protein with substitution of two selected residues, S564N and F577L, within the AcScH sequence is unable to rescue rRNA degradation and restricted viral replication in BM-N cells

Amino acid sequence alignments of the AcScH and BmScH regions revealed that 14 residues differ between the two sequences (see Fig. 1). A previous study showed that substitution of two amino acid residues, S564 and F577, within the AcScH sequence with the corresponding residues, N565 and L578, from BmScH is sufficient for AcMNPV to lethally infect *B. mori* larvae (Argaud et al., 1998). To determine whether these two residues within the AcScH sequence are specifically involved in rRNA degradation in BM-N cells, plasmids expressing Ac-P143 with a substitution of asparagine at S564 (Ac-P143(S564N)) or leucine at F577 (Ac-P143(F577L)), and a plasmid expressing Ac-P143 with both amino acid substitutions (Ac-P143(S564N/F577L)) were constructed and transfected into BM-N cells. At 0 and 72 h post-transfection, total RNAs were isolated from transfected BM-N cells and analyzed using the MultiNA system. When expressed in BM-N cells, each mutant Ac-P143 protein retained the capability to trigger rRNA degradation, as indicated by the weak, but clear detectable, band corresponding to ~1400-nt RNA fragments (Fig. 3A, lanes 3–8). These results indicated that one or more amino acid residues within AcScH sequence, other than S564 and F577, are involved in rRNA degradation in AcMNPV-infected BM-N cells.

Transfection-infection analysis was also performed to determine if BM-N cells expressing wt Ac-P143 protein or mutant Ac-P143 proteins with S564N, F577L, and S564N/F577L substitutions produce polyhedra (Fig. 3B). Quantification of polyhedra-producing cells revealed that very few or no BM-N cells expressing wt or the mutant Ac-P143 proteins produced detectable polyhedra (Fig. 3C).

Bm-P143 protein with a N565S/L578F substitution within the BmScH sequence triggers rRNA degradation and restricted viral replication

As the mutant Ac-P143 proteins with amino acid substitutions of S564N, F577L and S564N/F577L retained the capability to trigger rRNA degradation in BM-N cells (Fig. 3), it was concluded that the residues S564 and F577 are not essential for Ac-P143-triggered rRNA degradation in BM-N cells. To determine whether the S564 and F577 residues of Ac-P143 protein are involved in rRNA degradation, plasmids expressing Bm-P143 proteins with single and double amino acid