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The *Autographa californica* multiple nucleopolyhedrovirus *ac110* gene encodes a new *per os* infectivity factor

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ABSTRACT

The *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *ac110* gene is especially conserved in lepidopteran-specific baculoviruses and is uncharacterized. To investigate the role of *ac110* in the baculovirus life cycle, an *ac110*-knockout (vAc110KO) and a repair (vAc110:HA) viruses were constructed in this study. Budded virion production and occlusion body formation were unaffected in vAc110KO-transfected or infected Sf9 cells. Intrahemocoelic injection of budded virions of vAc110KO killed *Trichoplusia ni* larvae as efficiently as the repair or the wild-type viruses. However, *per os* inoculation of occlusion bodies of vAc110KO failed to establish infection in *T. ni* larvae, while the repair virus was as efficient as the wild-type virus. Treatment with calcofluor white, a reagent that damages the peritrophic membrane, did not rescue the oral infectivity of vAc110KO. These results suggested that Ac110 is a new *per os* infectivity factor that may play a role after occlusion-derived virions pass through the peritrophic membrane during oral infection.

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1. Introduction

Baculoviruses are a family of rod-shaped, enveloped, double-stranded DNA viruses, and their hosts are insects, including the order Lepidoptera, Diptera, and Hymenoptera. Two virion phenotypes, occlusion-derived virions (ODVs) and budded virions (BVs), are commonly produced in the baculovirus life cycle. Although BVs and ODVs are similar in nucleocapsid structure and carry identical genetic information, they differ in the composition of their envelopes and fulfill different roles in the virus life cycle. ODVs initiate primary infection in an insect host, while BVs are responsible for spreading infections among susceptible insect tissues (Rohrmann, 2013).

The site of primary baculovirus infection is the midgut of a susceptible insect. After being orally consumed by larvae, ODV-embedded occlusion bodies (OBs) reach the midgut and are dissolved by the alkaline environment. The first barrier that the released viruses encounter in the insect midgut is the peritrophic matrix (PM) (Rohrmann, 2013). The PM is composed of chitin, mucopolysaccharides, and proteins (Hegedus et al., 2009). It is thought that it provides protection to cells from damage caused by coarse food material and limits the access of microorganisms, while permitting transport of nutrients, water and minerals (Wang and Granados, 2000; Hegedus et al., 2009). Polyhedra are non-specifically contaminated with some proteinases that are likely a combination of enzymes derived from bacteria, the insect gut, and the virus (Rohrmann, 2013). Some of these proteinases are possible to promote the process of virus passing through the PM. The released ODVs pass through the PM and then bind to and fuse with the microvilli of midgut epithelial cells (Ohkawa et al., 2005). Microvilli are cylindrical projections of the apical plasma membrane with diameters of approximately 0.1 μm that are packed with bulky F-actin and cross filaments (Danielsen and Hansen, 2003). Currently, the basal transport mechanism of nucleocapsids via the bulky microvilli remains unknown. After the entry of nucleocapsids into the nucleus, viral genomes are released from the nucleocapsids and genes are transcribed in a complex, temporally regulated gene expression cascade, and progeny nucleocapsids assemble in the nucleus (Rohrmann, 2013). In the early infection phase, newly assembled nucleocapsids bud from the basal side of the midgut epithelial cells to form BVs, which spread the systemic infection to other tissues via the hemolymph (Granados and Lawler, 1981) and the tracheal system (Engelhard et al., 1994).

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The interaction of ODVs with midgut epithelial cells is saturable and competitive, suggesting that a specific receptor is involved (Horton and Burand, 1993). Treatments of midgut epithelial cells with phospholipase, neuraminidase, or β-glucosidase do not affect ODV binding (Horton and Burand, 1993). However, a profound decrease in ODV binding was observed after protease treatment, indicating that a protein receptor mediates this process (Horton and Burand, 1993). ODVs are structurally complex entities, and a cluster of these proteins is distributed in ODV envelopes (Braunagel et al., 2003; Braunagel and Summers, 2007; Hou et al., 2013; Xu et al., 2011; Wei et al., 2014). Some of the ODV envelope proteins are required for only the primary infection, and not for systemic infection. Thus, they are designated as *per os* infectivity factors (PIFs) (Kikhno et al., 2002; Rohrmann, 2013). The genome of *Autographa californica* multiple nucleopolyhedrovirus was the first baculovirus genome to be completely sequenced. AcMNPV belongs to the species *Autographa californica multiple nucleopolyhedrovirus* which is the type species of the genus *Alphabaculovirus* in the family *Baculoviridae* (Ayres et al., 1994). Currently, a total of eight genes in AcMNPV have been characterized as *pifs* which include *p74* or *pif-0* (*ac138*) (Faulkner et al., 1997), *pif-1* (*ac119*) (Kikhno et al., 2002), *pif-2* (*ac22*) (Fang et al., 2006; Pijlman et al., 2003), *pif-3* (*ac115*) (Ohkawa et al., 2005), *pif-4* (*ac96*) (Fang et al., 2009), *pif-5* (*ac148*) (Sparks et al., 2011; Xiang et al., 2011a), *pif-6* (*ac68*) (Nie et al., 2012), and *ac83* (Zhu et al., 2013). Additionally, *ac83* is a peculiar *pif* gene that functions in both *per os* infection and nucleocapsid assembly (Rohrmann, 2013; Zhu et al., 2013). When only the chitin-binding domain of Ac83 is deleted, the virus is unable to establish efficient *per os* infection, but it can infect the larvae via intrahemocoelic injection (Zhu et al., 2013). The *pif* genes are highly conserved in *Baculoviridae*; most are also found in other large invertebrate DNA viruses, such as salivary gland hypertrophy viruses (Garcia-Maruniak et al., 2009) and nudiviruses (Wang and Jehle, 2009).

PIF-1, PIF-2, and P74 are required for the binding of ODVs to epithelium, while PIF-3 and PIF-5 may participate in some critical downstream events, such as cell signaling and post-fusion events (Ohkawa et al., 2005; Sparks et al., 2011). P74 and PIF-5 interact with gut proteins with molecular masses of 35 kDa and 97 kDa, but these proteins have not yet been identified (Sparks et al., 2011; Zhou et al., 2005). PIF-1, PIF-2, and PIF-3 form a highly stable complex on the surface of ODV particles (Peng et al., 2012). PIF-4 interacts strongly with the complex, while P74 and Ac83 interact more loosely (Peng et al., 2012). Ac5, PIF-6, and Ac108 putatively associate with the complex, but PIF-5 is not present in the complex (Peng et al., 2012).

Recently, some less-conserved genes, such as the AcMNPV *odv-e66* (Xiang et al., 2011b), which is conserved in lepidopteran-specific baculoviruses (Garavaglia et al., 2012; van Oers and Vlak, 2007), and *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) *orf58* genes (*sf58*) (Simon et al., 2012), which is conserved in the genera *Alpha-*,*Beta-*, and *Gammabaculovirus* (Garavaglia et al., 2012; van Oers and Vlak, 2007), were found to play a role in oral infectivity. Also the lack of *Helicoverpa armigera* nucleopolyhedrovirus (HearSNPV) *orf100* (*ha100*) gene, which is conserved among group II *Alphabaculoviruses* (van Oers and Vlak, 2007), resulting in a 50% lethal concentration value that was higher and a median lethal time that was approximately 24 h longer than that of the control virus (Luo et al., 2011). *ac110* is conserved among the lepidopteran-specific baculoviruses (Garavaglia et al., 2012; van Oers and Vlak, 2007). The function of *ac110* remains unknown, although the *ac110* homolog in *Bombyx mori* nucleopolyhedrovirus, *BmP92a*, has been found to be nonessential for viral replication *in vitro* (Ono et al., 2012). In this study, an *ac110*-knockout virus (vAc110KO) was constructed to investigate the role *ac110* plays in the AcMNPV life cycle. Our results showed that *ac110* is a new *pif* gene that is required for oral infectivity and the PM is not the functional target of Ac110 during ODV infection.

2. Materials and methods

2.1. Virus, cells, and insects

The Sf9 cell line, which is derived from the fall armyworm *Spodoptera frugiperda*, was cultured at 27 °C in Grace's Insect medium (Invitrogen), which was supplemented with 10% FBS, penicillin (100 μg/ml), and streptomycin (30 μg/ml). The bacmid bMON14272 (Invitrogen) which contains an AcMNPV genome (Luckow et al., 1993) was propagated in *Escherichia coli* strain DH10B. The *Trichoplusia ni* larvae were reared on an artificial diet at 28 °C (Li et al., 2002).

2.2. Computer-assisted analysis

A similarity search of the Ac110 gene and its deduced amino acid sequences was performed against GenBank/EMBL, PIR and SWISS-PROT databases by using BLASTP, PSI-BLAST and FASTA programs (Altschul et al., 1997; Pearson, 1990). TM domain predictions were made using the TMPRED program (http://www.ch.embnet.org/software/TMPRED_form.html). Multiple sequence alignments were performed with CLUSTAL_X (Thompson et al., 1997) and edited with GeneDoc software (Nicholas et al., 1997).

2.3. Reverse transcription (RT)-PCR analysis

Sf9 cells (1×10^6 cells/35-mm-diameter well) were infected with AcMNPV at a multiplicity of infection (MOI) of 5 50% tissue culture infectious doses ($TCID_{50}$)/cell and harvested at indicated time points post infection (p.i.). Total RNA was isolated using RNeasy Protect Mini Kit (Qiagen). cDNA synthesis was performed using iScript cDNA Synthesis Kit (Bio-Rad). The *ac110*-specific primers Ac110RT1 (5′-ATGAAATATTTTCTGTCTGCTAC-3′) and Ac110RT2 (5′-TTATTTTAATTTGTGAACTCGTAC-3′) were used to detect the *ac110* transcripts. The transcripts of *vp39*, *gp64*, and *ie1* were detected by using the *vp39*-specific primers Vp39RT1 (5′-TGAGAGTTAATCGCTGCATTTTCGC-3′)/Vp39RT2 (5′-CTCTTGGTATAAAGTCGTTGCGC-3′), *gp64*-specific primers Gp64RT1 (5′-CGCTGGCATCTTTCCAACTGT-3′)/Gp64RT2 (5′-TTCATCGAGACGGCGTGAGTA-3′) and *ie1*-specific primers Ie1RT1 (5′-TTGTGATAAACAACCCAACGA-3′)/Ie1RT2 (5′-GTTAACGAGTTGACGCTTGC-3′).

2.4. Time course analysis of Ac110 expression

Sf9 cells (1×10^6 cells/35-mm-diameter well) were infected with vAc110:HA (see below) at an MOI of 5 $TCID_{50}$/cell. At the designated time points p.i., cells were harvested as described previously (Wu et al., 2008). Western blotting was performed using mouse monoclonal anti-HA antibody (1:3000; Convance) or anti-GP64 antibody (1:3000; eBioscience) as the primary antibody and a goat anti-mouse HRP antibody (1:5000; Amersham Biosciences) was used as the secondary antibody.

2.5. Construction of the recombinant AcMNPV bacmids

An *ac110*-knockout AcMNPV bacmid was generated via homologous recombination as previously described (Wu et al., 2006). In this case, the primer pair Ac110US1 (5′-GAGCTCCTCGATGGGACATTTGGGGT-3′, with the SacI site underlined)/Ac110US2 (5′-GGATCCCTATTGAATACGGTACGAGTTCACAAA-3′, with the BamHI site underlined) was used to PCR-amplify a 464-bp

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