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# *Autographa californica* multiple nucleopolyhedrovirus PK-1 is essential for nucleocapsid assembly

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ABSTRACT

PK-1 (Ac10) is a baculovirus-encoded serine/threonine kinase and its function is unclear. Our results showed that a *pk-1* knockout AcMNPV failed to produce infectious progeny, while the *pk-1* repair virus could rescue this defect. qPCR analysis demonstrated that *pk-1* deletion did not affect viral DNA replication. Analysis of the repaired recombinants with truncated *pk-1* mutants demonstrated that the catalytic domain of protein kinases of PK-1 was essential to viral infectivity. Moreover, those PK-1 mutants that could rescue the infectious BV production defect exhibited kinase activity *in vitro*. Therefore, it is suggested that the kinase activity of PK-1 is essential in regulating viral propagation. Electron microscopy revealed that *pk-1* deletion affected the formation of normal nucleocapsids. Masses of electron-lucent tubular structures were present in cell transfected with *pk-1* knockout bacmid. Therefore, PK-1 appears to phosphorylate some viral or cellular proteins that are essential for DNA packaging to regulate nucleocapsid assembly.



## Introduction

The family *Baculoviridae* consists of a diverse group of invertebrate-specific DNA viruses. Members of this family are characterized by having enveloped, rod-shaped virions with a circular double-stranded DNA genome (Miller, 1993). During the baculovirus infection cycle, progeny nucleocapsids have two fates: to be matured as budded virus (BV) or as occlusion-derived virus (ODV). BVs are produced during the early stage of baculovirus infection and obtain their envelopes as they bud off the plasma membranes of the infected cells. BVs mediate the systematic infection within the host (Keddie et al., 1989). ODVs are produced during the very late stage of baculovirus infection and embedded within a crystalline structure made up of polyhedrin proteins that form occlusion bodies (OBs). ODVs are responsible for horizontal transmission between insect hosts (Keddie et al., 1989). BV and ODV share dozens of the same proteins, in which some proteins are essential for nucleocapsid formation including P6.9 (Wang et al., 2010a), VP39 (Thiem and Miller, 1989), C42, AC142, EC27 (Vanarsdall et al., 2007) and 38 K (Wu et al., 2006). In *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), very late factor 1 (VLF-1) (Vanarsdall et al., 2006), VP1054 (Olszewski and Miller, 1997) and AC109 (EC43) (Lin et al., 2009) were also found to be the components of BV and ODV and were essential for nucleocapsid assembly. Nucleocapsid assembly takes place in the virogenic stroma of nucleus, indicating that viral genome is pre-packaged with the basic DNA binding protein P6.9 (Wang et al., 2010a), and the nucleoprotein complex is then inserted into a pre-formed tube-like capsid sheaths composed of VP39.

Protein phosphorylation, which is mediated by protein kinases (PKs), is one of the most important mechanisms for regulating protein activity and enabling the cell to respond to external signals. PKs catalyze the transfer of a phosphoryl group to a hydroxyl group on either Ser/Thr or Tyr residue (Hanks and Hunter, 1995). PKs regulate a wide range of eukaryotic cellular functions, including transcription, translation, cell division and differentiation (Graves and Krebs, 1999). Evidence has now accumulated to suggest that PKs are important in the virus life cycles, including steps in viral infection, uncoating, transcription and replication (Hui, 2002). PK-1 is the baculoviral encoded serine/threonine kinase (Possee et al., 1991; Reilly and Guarino, 1994). In AcMNPV, PK-1 is expressed in the late and very late phases of infection (Possee et al., 1991; Reilly and Guarino, 1994). In contrast, in *Choristoneura fumiferana* granulosis virus (CfGV) and *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV), PK-1 is expressed throughout the infection, suggesting it may be categorized as both an early and late baculovirus gene (Bischoff and Slavicek, 1994;

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Giannopoulos et al., 2005). PK-1 of AcMNPV has been shown to regulate very late *polh* promoter expression (Fan et al., 1996; Mishra et al., 2008a). Further studies indicated that PK-1 was a component of the very late gene transcription initiation complex of AcMNPV. And PK-1-mediated phosphorylation was a part of the regulatory mechanism to initiate transcription from the *polh* promoter (Mishra et al., 2008b). Fan et al. (1998) have shown using a yeast two-hybrid system that PK-1 interacts with an AcMNPV-encoded protein, PKIP, which stimulates the activity of PK-1. A temperature-sensitive mutation in the *pkip* gene caused AcMNPV to lose the ability to form plaques and occlusion bodies at the nonpermissive temperature (McLachlin et al., 1998). In *Bombyx mori* nucleopolyhedrovirus (BmNPV), a *pk-1* gene knockout virus failed to spread infection, but showed ability to express *egfp* in the transfection experiments (Ono et al., 2012). So, it is not very clear how PK-1 functions in early and late phases of infection.

In this study, we used an AcMNPV, one of the typical species of the *Baculoviridae*, to generate a *pk-1* knockout mutant by homologous recombination to determine the role of PK-1 in AcMNPV viral replication. Our data indicated that the PK-1 was essential for virion production, and its kinase activity seemed essential in regulating virion assembly.

## Results

### *Generation of recombinant AcMNPV bacmids*

To investigate the function of *pk-1* during the viral infection cycle, a bacmid containing a knockout in the *pk-1* gene was generated by the λ- red recombination system in *Escherichia coli*. In all the knockout candidates, nt 7043 to 7739 of the AcMNPV genome were successfully replaced by the *Cm* cassette (Fig. 1A), as all the PCR amplicons from catF/1020 were about 2.5 kb (expected size was 2452 bp) and amplicons from catR/1629 were nearly 2.7 kb (expected size was 2667 bp) (Fig. 1C).

To examine whether the *pk-1* deletion had any effect on virion morphogenesis and to facilitate observation of viral infection, the *polh* gene of AcMNPV and the *gfp* gene were inserted into the *polh* locus of vAc$^{pk\text{-}1\text{-}ko}$ via transposition. The resulting bacmid was named vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ (Fig. 1B). In order to assure the phenotype resulting from the deletion of *pk-1*, a repair bacmid, vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$, was generated in which the *pk-1* gene with the *pk-1* promoter and poly (A) signal, as well as *polh* and *gfp*, were inserted into vAc$^{pk\text{-}1\text{-}ko}$ *polh* locus by transposition (Fig. 1B). Additionally, a series of truncated *pk-1* fragments with the *pk-1* promoter and the OpIE2 poly (A) tail, as well as *polh* and *gfp*, were inserted into vAc$^{pk\text{-}1\text{-}ko}$ *polh* locus by transposition. The truncated fragments were Z1, Z2, Z3, Z4, Z5, Z6, Z7 and Z8 (Fig. 1B). The recombinants were named vAc$^{pk1\text{-}Z1}$, vAc$^{pk1\text{-}Z2}$, vAc$^{pk1\text{-}Z3}$, vAc$^{pk1\text{-}Z4}$, vAc$^{pk1\text{-}Z5}$, vAc$^{pk1\text{-}Z6}$, vAc$^{pk1\text{-}Z7}$ and vAc$^{pk1\text{-}Z8}$, respectively. As a positive control, vAc$^{PH\text{-}GFP}$ was generated by transposing the *polh* and *gfp* into the *polh* locus of the bMON14272 bacmid.

All constructs were confirmed by PCR analysis. An amplicon of 819 bp was amplified from vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$ or vAc$^{PH\text{-}GFP}$ by primers pk-1F/pk-1R, whereas no amplicon was produced from vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ (Fig. 1C), indicating that the *pk-1* gene was deleted in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$. No amplicon was amplified from vAc$^{PH\text{-}GFP}$ by primers catF/catR, but an amplicon of 1025 bp was produced in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ or vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$ (Fig. 1C), indicating that the *Cm* gene cassette was inserted in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ or vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$. No amplicon was produced by primers catF/1020 or catR/1629 in vAc$^{PH\text{-}GFP}$, whereas an amplicon of 2452 bp was produced using primers catF/1020 and that of 2667 bp was produced using primers catR/1629 in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ or vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$ (Fig. 1C), indicating the recombination junctions of *Cm* gene cassette with flanking regions of *pk-1* gene were generated in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$ and vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$.

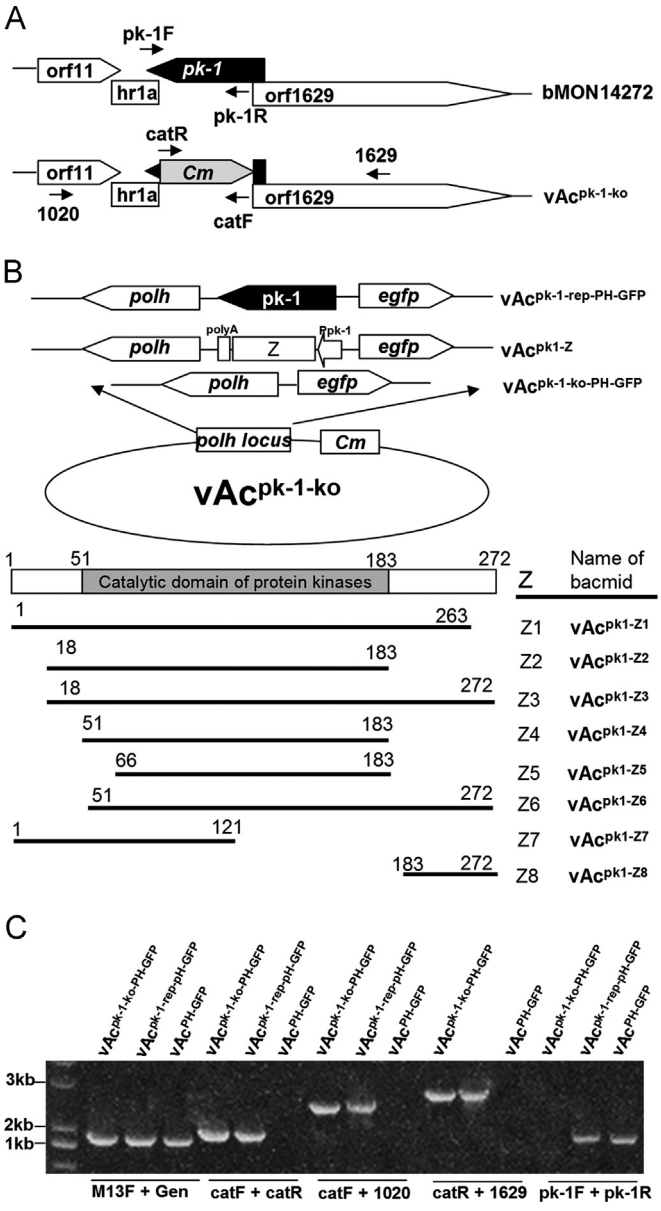


**Fig. 1.** Generation of recombinant AcMNPV bacmids. (A) Diagram of *pk-1* knockout. The C-terminal coding region (127 to 819 bp) of *pk-1* on AcMNPV bacmid bMON14272 was replaced with a *Cm* expression cassette. The knockout bacmid was named vAc$^{pk\text{-}1\text{-}ko}$. Four sets of primers, pk-1F/pk-1R, catR/catF, 1020/catE and 1629/catR, are labeled with arrows at their corresponding loci, which were used for PCR confirmation in *pk-1* knockout. (B) The schematic diagram of the recombinant bacmids derived from vAc$^{pk\text{-}1\text{-}ko}$. All of transposed bacmids possess a *gfp* gene and a *polh* gene. vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$ was inserted a wild type *pk-1* expression cassette driven by *pk-1*'s native promoter and polyA tail. vAc$^{pk1\text{-}Z}$ was inserted Z, which is represented by the truncated *pk-1* mutants, driven by *pk-1*'s native promoter and OpIE2 polyA tail. The designations for *pk-1* mutants are indicated under the "Z", and the names of the corresponding recombinant repair bacmids are shown on the right. The delete sketch map at the top represents the PKc domain (51 to 183 aa). (C) PCR analysis of the presence or absence of sequence modifications in vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$, vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$, and vAc$^{PH\text{-}GFP}$. The virus templates are shown above each lane, and the primer pairs used are shown below.

### *PK-1 is essential for viral propagation*

To examine the effect of *pk-1* deletion on the viral propagation, *Sf*9 cells were transfected with vAc$^{pk\text{-}1\text{-}ko\text{-}PH\text{-}GFP}$, vAc$^{pk\text{-}1\text{-}rep\text{-}PH\text{-}GFP}$ and vAc$^{PH\text{-}GFP}$, respectively. The GFP fluorescence cells were