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Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene

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Abstract

Autographa californica multiple nucleopolyhedrovirus (Ac*M*NPV) encodes two proteins that possess properties typical of single-stranded DNA-binding proteins (SSBs), late expression factor-3 (LEF-3), and a protein referred to as DNA-binding protein (DBP). Whereas LEF-3 is a multi-functional protein essential for viral DNA replication, transporting helicase into the nucleus, and forms a stable complex with the baculovirus alkaline nuclease, the role for DBP in baculovirus replication remains unclear. Therefore, to better understand the functional role of DBP in viral replication, a DBP knockout virus was generated from an Ac*M*NPV bacmid and analyzed. The results of a growth curve analysis indicated that the *dbp* knockout construct was unable to produce budded virus indicating that *dbp* is essential. The lack of DBP does not cause a general shutdown of the expression of viral genes, as was revealed by accumulation of early (LEF-3), late (VP39), and very late (P10) proteins in cells transfected with the *dbp* knockout construct. To investigate the role of DBP in DNA replication, a real-time PCR-based assay was employed and showed that, although viral DNA synthesis occurred in cells transfected with the *dbp* knockout, the levels were less than that of the control virus suggesting that DBP is required for normal levels of DNA synthesis or for stability of nascent viral DNA. In addition, analysis of the viral DNA replicated by the *dbp* knockout by using field inversion gel electrophoresis failed to detect the presence of genome-length DNA. Furthermore, analysis of DBP from infected cells indicated that similar to LEF-3, DBP was tightly bound to viral chromatin. Assessment of the cellular localization of DBP relative to replicated viral DNA by immunoelectron microscopy indicated that, at 24 h post-infection, DBP co-localized with nascent DNA at distinct electron-dense regions within the nucleus. Finally, immunoelectron microscopic analysis of cells transfected with the *dbp* knockout revealed that DBP is required for the production of normal-appearing nucleocapsids and for the generation of the virogenic stroma.

Keywords: Baculovirus; Single-strand DNA binding protein; SSB; Virogenic stroma; DNA replication; Nucleocapsid

Introduction

The Baculoviridae consists of a diverse family of rod shaped viruses that contain circular covalently closed dsDNA genomes that range in size from 80 to 180 kbp. Baculovirus infections are restricted to invertebrates and the most well studied example is the *Autographa californica* multiple nucleopolyhedrovirus (Ac*M*NPV) which is pathogenic for several species of Lepidoptera. Upon entry of Ac*M*NPV into a susceptible host cell, replication occurs in the nucleus to generate two virus types. Budded virus (BV) is produced from nucleocapsids that become enveloped during egress through the plasma membrane that has been modified by the viral fusion protein GP64. In contrast, occlusion-derived virions (ODV) are produced from nucleocapsids that remain in the nucleus where they are enveloped prior to becoming occluded within a crystalline matrix comprised of polyhedrin. Whereas BV is associated with systemic infection, ODV mediates lateral transmission between insects when released into the environment upon death of the host.

It has been determined through the use of a transient replication assay that six baculovirus gene products are required for viral DNA replication (Kool et al., 1994; Todd et al., 1995). These include an activator of transcription (*ie-1*), a helicase (*p143*), a DNA polymerase (*dnapol*), late expression factor-1

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(*lef-1*) that functions as a primase, *lef-2* that serves as a primase accessory factor, and *lef-3*, a single-stranded DNA-binding protein. More recently, the *lef-11* gene product was shown to be required for replication of bacmid DNA in tissue culture (Lin and Blissard, 2002).

Previously, a search for additional DNA-interacting proteins from BmN cells infected with *Bombyx mori* nucleopolydedrovirus (BmNPV) identified a 37-kDa protein designated as DNA-binding protein, or DBP (Mikhailov et al., 1998). Interestingly, subsequent biochemical analysis of DBP after purification to near homogeneity indicated that it possessed properties of a bona fide single-stranded DNA-binding protein (SSB). These properties include a much higher affinity for ssDNA over dsDNA, the ability to protect bound DNA substrates from exonuclease digestion, and the ability to unwind duplex DNA substrates in a dose-dependant manner without ATP (Mikhailov et al., 1998). Proteolytic digestion of the purified protein followed by mass spectrometry analysis indicated that DBP was encoded by ORF16 of BmNPV, and homologs of this gene have been identified in all baculovirus genomes sequenced, except for one infectious for a dipteran, that also lacks an identifiable homolog of *lef-3* (Okano et al., 2006). In addition, analysis of the temporal expression pattern of DBP in BmNPV-infected cells indicated that it is expressed as an early gene, initially detectable by 4 h post-infection (h.p.i.) and peaking at 14 h.p.i. and is not a virion structural protein (Okano et al., 1999). Using confocal microscopy to characterize the localization of DBP in infected cells, it was shown that at early times, DBP colocalizes with viral DNA, LEF-3, and IE-1; however, by 14 h.p.i., DBP became more diffuse and did not appear to colocalize with IE-1 or LEF-3 (Okano et al., 1999). The location of DBP at the viral DNA replication sites was confirmed for Ac*M*NPV, a close relative of BmNPV (Mainz et al., 2002). A putative homologous region (*hr*) replication origin and two replication factors, IE-1 and helicase, were found to promote localization of both baculovirus SSBs, LEF-3 and DBP, to subnuclear foci (Nagamine et al., 2006). These observations suggest that DBP may be closely associated with replicating DNA during the early stages of infection, or could possibly function in DNA processing during the later stages of infection.

The observation that baculoviruses encode two single-stranded DNA-binding proteins raises interesting questions regarding their roles in DNA replication and processing. In this report we describe the construction of an Ac*M*NPV bacmid lacking the *dbp* gene (Ac*M*NPV ORF25) and the results of a series of experiments designed to gain insight into the function of DBP during infection.

Results

Analysis of budded virus production and gene expression

To determine whether *dbp* was required for BV production, a growth curve analysis was performed to monitor the amount of budded virus produced from cells transfected with the *dbp* knockout (*dbp*-KO), *dbp* repaired (*dbp*-rep), and control (AcGUS), (Vanarsdall et al., 2004) bacmids. For these analyses, cell supernatants were collected every 24 h up to 120 h post-transfection (h.p.t.) and titered by end-point dilution assays. As shown in Fig. 1, no infectious BV was detected in supernatants collected from cells transfected with the *dbp*-KO bacmid at any time-point post-transfection. In contrast, beginning at 24 h.p.t. and continuing to 120 h.p.t., the BV titer from cells transfected with the *dbp* repair virus was similar to the level of BV produced from cells transfected with the infectious control bacmid, indicating that re-inserting the *dbp* ORF at the polyhedrin locus of the original *dbp* knockout was sufficient to restore DBP expression (Fig. 2A) and repair the non-infectious phenotype (Fig. 1) and confirmed that the lack of BV production in the null mutant is directly due to deletion of the *dbp* gene.

To rule out the possibility that the lack of BV production in cells transfected with the *dbp*-KO bacmid was due to a defect in viral gene expression, we investigated whether DBP was necessary for the expression of different classes of viral proteins. The accumulation of early (LEF-3), late (VP39), and very late (P10) proteins in cells transfected with the *dbp*-KO bacmid was monitored at different times post transfection (Fig. 2). The cell extracts were subjected to SDS-PAGE, and the presence of LEF-3 and VP39 was determined by using Western blotting and specific antibodies (panels B and C), whereas the abundant very late protein P10 was directly visualized by staining with Coomassie blue (panel D). The results of this analysis showed that all these proteins were synthesized in the cells lacking DBP (Figs. 2B–D). The more robust expression of P10 by the *dbp* repair construct is likely due to the fact that it is an infectious virus. These data indicate that DBP is not essential for synthesis of viral products and confirm previous

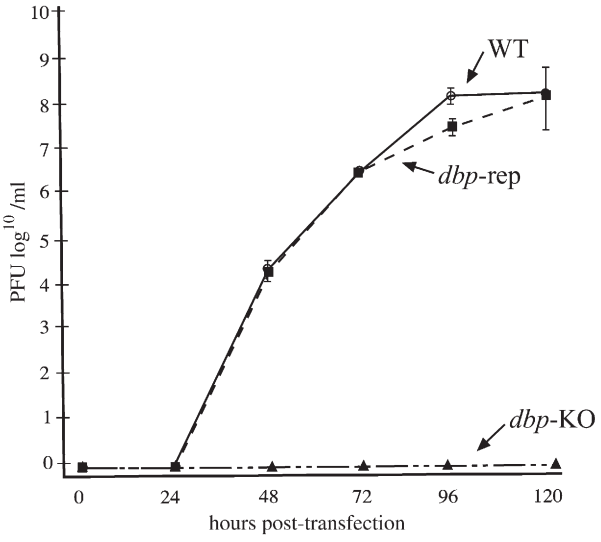

Fig. 1. Analysis of budded virus production from bacmid transfected cells. At the indicated time-points, the supernatants from Sf-9 cells transfected with equimolar amounts of the *dbp* knockout bacmid (*dbp*-KO), the *dbp* repair bacmid (*dbp*-rep), or a control bacmid (AcGUS) (WT) were removed and the titers determined by $TCID_{50}$ end-point dilution assays. The points indicate averages from transfections performed in triplicate and error bars represent standard deviations.