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AcMNPV AC16 (DA26, BV/ODV-E26) regulates the levels of IE0 and IE1 and binds to both proteins via a domain located within the acidic transcriptional activation domain

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ABSTRACT

IE0 and IE1 are the primary viral regulatory proteins of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) involved in the transactivation of early genes, stimulation of late gene expression, and viral DNA replication. The protein interactions required for IE0 or IE1 to achieve these varied roles are not well defined, so to identify proteins that interact with IE0 and IE1, tandem affinity purification (TAP) and LC-MS/MS was used. Analysis of purified proteins identified AC16 (DA26, BV/ODV-E26) from TAP tagged IE0 virus infected Sf9 cells. Co-immunoprecipitation confirmed that AC16 interacts with both IE0 and IE1 and yeast 2-hybrid analysis mapped the domain required for interaction with AC16. Mutation of the AC16 binding domain enhanced BV production by viruses expressing only IE0 but had no effect if only IE1 is expressed. An *ac16* deletion virus was constructed and was shown not to affect the temporal expression of IE0 and IE1; however the relative level of IE0 to IE1 was significantly increased.

Introduction

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is the type species of the Baculoviridae Alphabaculovirus genus. The baculoviridae consists of a large group of enveloped double stranded DNA viruses. Baculovirus genes can be divided into immediate early, early, late and very late based on the expression cascade (Kelly and Lescott, 1981; Miller, 1997). The primary AcMNPV transregulatory proteins are the immediate early IE0 and IE1 which are produced from the only known AcMNPV spliced gene complex. The *ie0* mRNA consists of two exons that initiates from the early promoter of a*c141*. Exon 1 of *ie0* consists of 114 bp of *ac141* that are spliced to the 5′ end of the *ie1* mRNA after excision of a 4.2 kb intron (Chisholm and Henner, 1988). The *ie1* mRNA is not spliced and consists of only exon 2 and initiates from the early promoter of *ac147*. Both *ie0* and *ie1* are transcribed and translated immediately upon infection but peak at different times post infection (pi). The steady state levels of IE0 peak prior to viral DNA replication at about 3–6 h pi, whereas IE1 keeps accumulating until very late times pi (Huijskens et al., 2004). Interestingly, expression of *ie0* mRNA results in both IE0 and IE1 being translated from the *ie0* mRNA due to internal translation initiation (Theilmann et al., 2001). The amino acid sequence of IE0 includes 54 extra amino acids at N-terminal of IE1 that are derived from the *ac141* ORF and the *ie1* 5′ untranslated region.

IE0 and IE1 appear to have primary functions at different stages of the viral life cycle. Both IE1 and IE0 are capable of transactivating viral early genes and stimulating late gene expression (Huijskens et al., 2004; Kovacs et al., 1991; Theilmann et al., 2001). Either IE0 or IE1 can support viral replication independently however both are required for a wild type phenotype (Stewart et al., 2005). Reduced expression level of IE0 enables AcMNPV to replicate in the normally non-permissive SL2 cells therefore suggesting that IE0 might influence host range determination (Lu et al., 2003). However it's not clear how IE0 and IE1 orchestrate their functional similarities or differences and little is known about the identity of the proteins with which IE0 or IE1 interact. AcMNPV IE1 is well known as one of the six essential genes along with *dna polymerase*, *lef-1*, *lef-2*, *lef-3*, and *helicase* required for the viral DNA replication in transient replication assays (Kool et al., 1994). It has also been shown that BmNPV IE1 co-localizes with DBP and LEF3 in the nuclear structures that are believed to be viral replication factories (Kawasaki et al., 2004; Okano et al., 1999). Ito et al. (2004) found that AcMNPV IE1, LEF3 and P143 bind to closely linked sites on viral DNA *in vivo* using chromatin immunoprecipitation and suggested these proteins may form replication complex in infected

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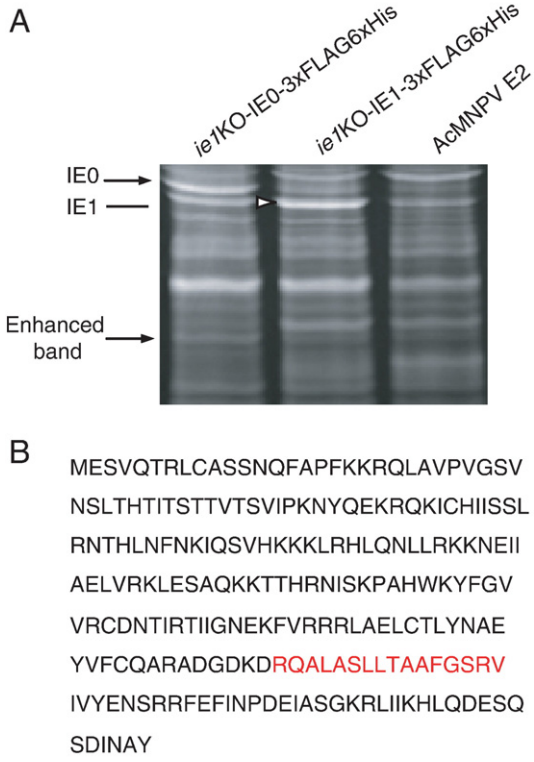

Fig. 1. AC16 co-purifies with TAP tagged IE0. (A) Sf9 cells were infected with viruses expressing TAP tagged IE0 (*ie1*KO-IE0-3xFLAG6xHis), IE1 (*ie1*KO-IE1-3xFLAG6xHis) or with the control virus AcMNPV-E2 that expresses untagged IE0 and IE1 and collected for TAP at 12 hpi. Complexes were affinity purified and separated on gradient SDS-PAGE gel (4–12%) and stained with SYPRO Ruby. A protein was found to be enhanced in proteins purified from *ie1*KO-IE0-3xFLAG6xHis infected cells (arrow). (B) Amino acid sequence of AC16 and the LC-MS/MS analysis of the enhanced band identified a peptide homologous to the sequences shown in red.

cells. Besides the proteins involved in viral DNA replication, *Bombyx mori* NPV (BmNPV) IE1 was also found to co-localize in infected insect cells and interact in yeast 2-hybrid assays with BmNPV BM8 (Imai et al., 2004; Kang et al., 2005). BM8 is the homolog of AcMNPV AC16, which is one of the 17 Group I NPV specific genes (Herniou et al., 2001). Further identification of proteins that interact with IE0 and IE1 and elucidation of the biological relevance of the interaction is needed to enable the complete understanding of the essential roles that IE0 and IE1 play during the viral life cycle.

In this study tandem affinity purification (TAP) has been used to identify proteins that interact with TAP-tagged AcMNPV IE0 and IE1. Using this approach AcMNPV AC16 (also known as DA26, BV/ODV-E26) (Beniya et al., 1998) was identified. The interaction was confirmed by reciprocal immunoprecipitation to pull down both IE0 and IE1 along with HA tagged AC16. Using yeast 2-hybrid assays the AC16 interaction domain of IE0 and IE1 was mapped to the acidic activation domain (AAD). Viruses were constructed that expressed only IE0 or IE1 with mutated AC16 interaction domains and the impact on the virus replication cycle was analyzed. In addition, the impact of *ac16* deletion on the virus replication was also determined.

Results

Identification of IE0 and IE1 interacting proteins by tandem affinity purification

AcMNPV IE0 and IE1 are the primary viral transregulatory proteins and identifying other proteins that complex with them is essential to understand how AcMNPV replicates. TAP purification has been shown to enable the purification of large transcriptional complexes (Rigaut et al., 1999) and therefore we attempted to use this method with IE0 and IE1. To enable the TAP purification for the identification of interaction partners of AcMNPV IE0 and IE1, bacmid derived viruses that express IE0 or IE1 tagged with 3xFLAG-6xHis at the C-terminus (*ie1KO*-IE0-3xFLAG6xHis and *ie1KO*-IE1-3xFLAG6xHis respectively) were made using a previously constructed *ie1* knockout (KO) virus (Stewart et al., 2005). The IE0 and IE1 TAP tagged viruses and control virus AcMNPV E2 were used to infect Sf9 cells. At 12 h post infection (hpi) and 24 hpi, cells were collected and TAP purification was performed using anti-FLAG beads followed by Ni-NTA beads. The purified proteins were separated on a gradient gel (4–12%) and stained by SYPRO ruby staining. There were significant background bands using this approach and very few clear differences were observed, however a protein band between 32.5 kDa to 47.5 kDa was enhanced in the proteins bound to IE0-3xFLAG6xHis at both 12 hpi (Fig. 1A) and 24 hpi (data not shown). The band was excised from the gel of 12 hpi samples and subjected to LC-MS/MS for protein identification. One peptide was identified matching the viral protein AC16 (Fig. 1B). This was surprising as the predicted molecular weight of AC16 is 26 kDa, however, it is consistent with previous results (Beniya et al., 1998; Braunagel et al., in press; Burks et al., 2007) which have shown that higher molecular weight forms of AC16 are observed in infected cells. In addition, we also observed higher molecular weight forms of AC16 on Western blots (data not shown). The association of AC16 with IE0 also agrees with results obtained with the related virus BmNPV, which showed that the homolog of AC16, BM8, interacts with BmNPV IE1.

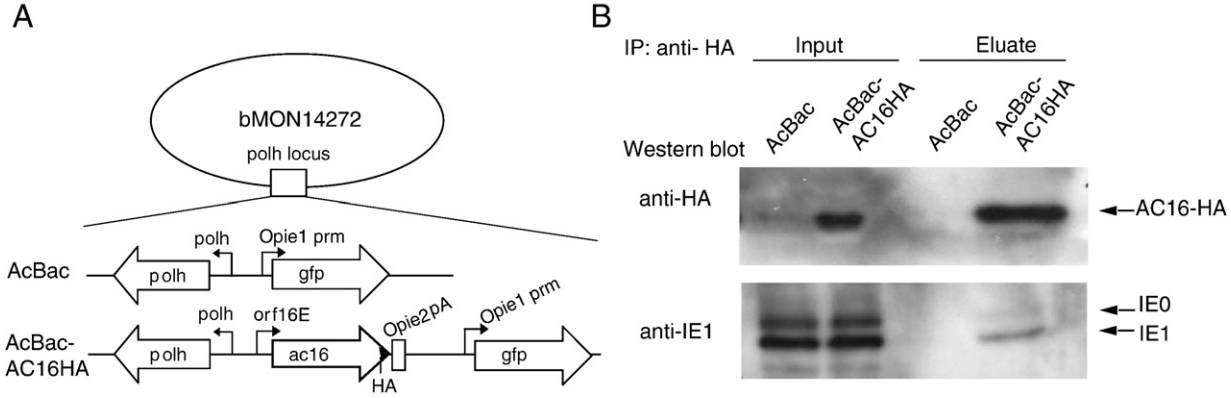

Fig. 2. IE0 and IE1 co-immunoprecipitate with AC16. Protein extracts of Sf9 cells infected with AcBac-AC16HA expressing HA tagged AC16 or the control virus AcBac expressing non-tagged AC16, respectively, were immunoprecipitated with anti-HA agarose beads at 18 hpi. SDS-PAGE and Western blots were used to analyze the precipitated proteins. AC16 and IE0 and IE1 were detected using anti-HA or anti-IE1 antibodies respectively. Top panel shows AC16HA, bottom panel shows IE0 and IE1. The input and eluate lanes represent 0.5% and 30% of the total input and eluate samples respectively.