The acidic activation domains of the baculovirus transactivators IE1 and IE0 are functional for transcriptional activation in both insect and mammalian cells

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The acidic activation domains (AADs) of the baculovirus transactivators IE1 and IE0 are essential for transcriptional transactivation. To compare the relative transcriptional activation potentials of IE1 and IE0 AADs of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Orgyia pseudotsugata* MNPV (OpMNPV), we constructed two ecdysone receptor (EcR)-based inducible expression systems to analyse six baculovirus AADs in two insect cell lines (Ld652Y and Sf9) and two mammalian cell lines (NIH-3T3 and CHO). For insect cell expression, the AADs were fused to the C, D, E and F domains of the spruce budworm *Choristoneura fumiferana* EcR. For mammalian cell expression the AADs were fused to the E and F domains of mammalian *Mus musculus* retinoid X receptor. In Ld652Y and Sf9 cells, chimeric proteins containing the AcMNPV AADs activated gene expression to higher levels than those containing the OpMNPV AADs. In NIH-3T3 cells, chimeras containing AcMNPV IE1 and IE0 AADs consistently activated gene expression to higher levels than the archetypal mammalian herpesvirus VP16 AAD. In contrast, OpMNPV AADs only activated expression by 5–15% relative to the VP16 AAD. In CHO cells, both AcMNPV and OpMNPV AADs exhibited intermediate transactivation levels relative to VP16 AAD. These results show that the baculovirus AADs are functional for transcriptional activation in mammalian cells and that AcMNPV AADs generally appear to be more potent than OpMNPV AADs in both insect and mammalian cells.

**INTRODUCTION**

The baculovirus transcriptional transactivator, IE1, plays a central role in regulating viral gene expression in infected insect cells. IE1 is a multifunctional protein; it is a highly potent transactivator of viral early and late promoters by enhancer-dependent and -independent mechanisms and in addition can negatively regulate transcription from specific promoters. IE1 is also essential for transient viral DNA replication and directly affects viral growth (Friesen, 1997; Lu et al., 1997).

IE1 has a modular structure that consists of separable domains. The N-terminal 132 amino acids of *Orgyia pseudotsugata* multiple nucleopolyhedrovirus (OpMNPV) IE1 and 151 amino acids of *Autographa californica* MNPV (AcMNPV) IE1 have been shown to contain an acidic activation domain (AAD) that is essential for transcriptional activation (Forsythe et al., 1998; Kovacs et al., 1992; Rodems et al., 1997; Slack & Blissard, 1997; Theilmann & Stewart, 1991). In addition, the N-terminal AAD of AcMNPV IE1 activates transcription when fused to a heterologous DNA-binding domain (Rodems et al., 1997; Slack & Blissard, 1997). Forsythe et al. (1998) performed a mutational analysis of OpMNPV IE1 AAD and showed that it comprised two activation subdomains. More recently, Pathakamuri & Theilmann (2002) showed that the AAD of OpMNPV IE1 also contains an essential DNA replication subdomain that is separate from the transcriptional activation domain.

The *ie1* gene is the only known baculovirus gene that is spliced, resulting in a transcript that encodes a second transactivator, IE0. The splicing event adds 54 and 35 amino acids to the N-terminal AAD of IE1 in AcMNPV and OpMNPV, respectively, to produce IE0 (Chisholm & Henner, 1988; Kovacs et al., 1991a, b; Theilmann & Stewart, 1991; Theilmann et al., 2001). The data from several groups indicate that the
additional residues added at the N terminus of IE0 may confer different transcriptional regulatory properties from IE1 (Kovacs et al., 1991b; Pearson & Rohrmann, 1997; Theilmann et al., 2001). In addition, it has recently been shown that ie0 mRNA has novel translational properties, which favour translation from internal start codons resulting in ie0 producing both IE1 and IE0 (Theilmann et al., 2001). An ie0 mutant that is translated only as IE0 was shown to transactivate gene expression at a level 14-fold higher than IE1 (Theilmann et al., 2001). This indicates that the additional amino acids at the N terminus of IE0 modify the transcriptional activation of this protein relative to IE1. Therefore, co-expression of IE1 and IE0 in infected cells may permit subtle regulation of specific sets of viral genes.

It is well known that the native AADs from the mammalian-derived herpesvirus VP16 and the yeast protein GAL4 are changeable and functional for transcriptional activation when fused to heterologous proteins in many mammalian cells (Li et al., 1998; Murakami & Ito, 1999; Ptashne, 1988). Forsythe et al. (1998) showed that the AAD of OpMNPV IE1 can be functionally replaced by VP16 in insect cells. This suggests that baculovirus AADs may be able to interact with the general transcription machinery and may function similarly to VP16 and GAL4 and function in many cell types including mammalian cells.

To compare the relative transcriptional activation potential of the essential AAD domains of IE1 and IE0 of AcMNPV and OpMNPV in both insect and mammalian cells, we constructed two ecdysone receptor (EcR)-based inducible expression systems to analyse six baculovirus AADs in two insect cell lines, Ld652Y and Sf9, and in two mammalian cell lines, NIH-3T3 and CHO. For insect cell expression, the AADs were fused to the C, D, E and F domains of the spruce budworm Choristoneura fumiferana EcR (CfEcR). For mammalian cell expression, the AADs were fused to the E and F domains of mammalian Mus musculus retinoid X receptor (MmRXR). Transcriptional activation by the resulting CfEcR and MmRXR chimeras was measured with reporter gene constructs containing the Drosophila melanogaster hsp27 ecdysone response element (7×EcRE) followed by a minimal OpMNPV ie2 basal promoter containing a TATA box and early mRNA transcriptional start site CAGT. For insect cell expression, the AADs were fused to the C, D, E and F domains of the spruce budworm Choristoneura fumiferana EcR (CfEcR). For mammalian cell expression, the AADs were fused to the E and F domains of mammalian Mus musculus retinoid X receptor (MmRXR). Transcriptional activation by the resulting CfEcR and MmRXR chimeras was measured with reporter gene constructs containing the Drosophila melanogaster hsp27 ecdysone response element or the Saccharomyces cerevisiae GAL4 response element in insect and mammalian cells, respectively. Our results showed that baculovirus AADs are highly functional for transcriptional activation in both mammalian and insect cells and that AcMNPV AADs are generally more potent than OpMNPV AADs.

**METHODS**

**Plasmids**

**Reporters.** EcR-CAT (Fig. 1A) was constructed as follows. An EcoRV–Xbal fragment containing the CAT open reading frame was PCR amplified with upper primer 5′-CTTCTAGACCGGTGGCGCA-CGGCGTGAAG-3′ and lower primer 5′-ATGATATCTTAAATACAGCCGCAACGATCTGGG-3′ using OpI1ECAT as a template (Theilmann & Stewart, 1993). This fragment was cloned into EcoRV/Xbal-digested pZeoS-9 (Invitrogen) to give pZeoSyn-1. A

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**Fig. 1.** Construction of an insect cell EcR-based inducible gene expression system. (A) Schematic diagram of a reporter EcR-E-CAT, which contains the CAT gene under the transcriptional control of seven copies of the Drosophila melanogaster hsp27 ecdysone response element (7×EcRE) followed by a minimal OpMNPV ie2 basal promoter containing a TATA box and early mRNA transcriptional start site CAGT. (B) Schematic diagram of plasmids that express chimeric CfEcRs. The AADs from AcMNPV and OpMNPV IE1, IE0 and IE0 from herpesvirus VP16 were fused to the C, D, E and F domains of CfEcR. Expression of the chimeric CfEcRs was under the control of the AcMNPV ie1 promoter.

**C. fumiferana** ecdysone receptors containing variable acidic activation domains. A basic vector pAct1E-ES8-CfEcR was prepared as follows. A fragment containing the AcMNPV ie1 promoter was amplified with upper primer 285 (5′-AGCCATATGCTGCTGACACACTATTA-3′) and lower primer 285 (5′-ATGGATATTGACTCTACTTGTTGTGGG-3′) using EcoRI B fragment of AcMNPV viral DNA as a template. The PCR fragment was cloned into NdeI/EcoRI-digested CfEcR-VP16 (Palli et al., 2003) to give

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pAcIE1-ESB-CfEcR. The vector contains unique EcoRI, Smal and BamHI sites for cloning of activation domains into pAcIE1-ESB-CfEcR for producing chimeric CfEcRs.

The CfEcR chimera expression plasmids are shown in Fig. 1(B). The cDNA encoding the C, D and E domains of CfEcR was fused to heterologous AADs from AcMNPV and OpMNPV IE1 and IE0 and from VP16 to produce chimeric CfEcRs. These chimeric CfEcRs were controlled by the AcMNPV 1ei promoter. The chimeric CfEcR expression construct containing VP16 AAD, CfEcR-VP16, has been previously described (Palli et al., 2003). To construct chimeric CfEcRs containing baculovirus AADs, the baculovirus AADs were amplified by PCR using primers with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were cloned into pAcIE1-ESB-CfEcR digested with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were cloned into pAcIE1-ESB-CfEcR digested with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were verified by sequence analysis.

M. musculus retinoid X receptors containing variable acidic activation domains. The M. musculus retinoid X receptor (MrXR) chimeric expression plasmids for mammalian cells are shown in Fig. 5(B). The cDNA coding the E and F domains of MmRXR was fused to heterologous AADs from AcMNPV and OpMNPV IE1 and IE0 and from VP16 to generate MmRXR chimeras, controlled by the SV40 promoter. Chimeric MmRXR containing baculovirus AADs, the baculovirus AADs were amplified by PCR using primers with Bacillus AADs, the baculovirus AADs were amplified by PCR using primers with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were cloned into pAcIE1-ESB-CfEcR digested with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were cloned into pAcIE1-ESB-CfEcR digested with EcoRI and BamHI at the 5′ and 3′ ends, respectively. The PCR products were verified by sequence analysis.

Tissue culture and transient transfection. Insect cell line Spodoptera frugiperda IPLB-Sf21-AC. The cell line was cultured in suspension at 27 °C in TC100 medium supplemented with 10% fetal bovine serum (complete media). Mammalian NIH-3T3 (mouse embryo fibroblast, ATCC no. CRL-1658) and CHO (Chinese hamster ovary, ATCC no. CCL-61) cells were maintained at 37 °C in Dulbecco’s modified Eagle’s medium (DMEM) and F-12 nutrient mixture medium (HAM), respectively, supplemented with 10% fetal calf serum. All media contained 50 μg of the antibiotic gentamicin sulfate ml−1 (Life Technologies).

Lipopectin was prepared as previously described (Campbell, 1995). The optimal ratio of lipopectin/DNA was measured by titration with a green fluorescent protein-expressing plasmid. Transient transfection was performed in duplicate in six-well plates by transferring 0.5 μg reporter and 1-0 μg receptor(s) plasmids into 1 x 10⁶ cells per well. The plasmid PBS+ was added to transfection mixtures if it was needed to ensure that an equivalent mass of DNA was delivered to the cells. For insect cells, the media containing the DNA and lipopectin mixture were removed after a 5 h incubation period at 27 °C and replaced with fresh medium. The cells were then cultured at 27 °C for 12 h and the medium was replaced with fresh medium with or without 0.5 μM RG-102240, a synthetic ecdysteroid analogue, also known as GSE [N-(1,1-dimethylallyl)-N’, (2-ethyl-3-methoxybenzoyl)-3,5-dimethylbenzohydrazide] (Palli et al., 2003). For mammalian cells, the medium containing DNA and lipopectin was removed after a 17 h incubation period at 37 °C and replaced with fresh medium with or without 10 μM MG-102240. Cells were harvested by scraping from the dishes at 48 h post-transfection for insect cells and 72 h for mammalian cells and used for a CAT activity assay and Western blotting.

Chloramphenicol acetyltransferase (CAT) assay. Harvested cells were pelleted and the media removed. The pellet was resuspended in 100 μl 250 mM Tris/HCl, pH 7.8. Cells were lysed by freeze-thawing three times and cellular deacetylases were inactivated at 65 °C for 15 min, followed by centrifugation at 10,000 r.p.m. for 1 min. Cell extracts were titrated to determine the appropriate quantity of extract to use to ensure a linear response in the assay. For the assay, an equal volume of cell extract (2.5-25 μl) for each sample was added to CAT assay buffer (6-25 mM chloramphenicol, 160 mM Tris/HCl, pH 7-8, 3-2 μM acetyl coenzyme A (Sigma) and 0-025 μg/ml (125 pmol) 1H]acetyl coenzyme A (CAT Assay Grade; New England Nuclear) in a total volume of 125 μl. The mixture was overlaid with 3 ml toluene-based scintillation fluid (Econoflour-2; Packard Bioscience Co.). All experiments were repeated at least two times, each in duplicate.

Western blots. Proteins were separated by SDS-PAGE using a Bio-Rad Mini-Prottein II apparatus and transferred to Millipore Immobilon membrane using liquid-transfer apparatus. Three primary antibodies were used in this study: (i) EcR antibody: mouse monoclonal antibody (mAb) MsEcR-9B9 (Developmental Studies Hybridoma Bank, University of Iowa); (ii) AcMNPV IE1 antibody: mouse mAb IE1B7 (Ross & Guarino, 1997); (iii) OpMNPV IE1 antibody: mouse mAb IE1-10 (Theilmann & Stewart, 1993). The MmRXR antibody RRHa (AN197) was obtained from Santa Cruz Biotechnology. After incubation with a peroxidase-conjugated secondary antibody, signals were detected by Western blotting with enhanced chemiluminescence (Amersham) and quantified with ImageQuant software (Molecular Dynamics).
RESULTS

Construction of CfEcR chimeras containing baculovirus IE1 and IE0 AAD

To compare IE1 and IE0 AADs in insect cells, chimeric CfEcR constructs were made. The AADs from AcMNPV and OpMNPV IE1 and IE0 were fused to the C, D, E and F domains of CfEcR to produce four chimeric CfEcR constructs, CfEcR-AcIE1, CfEcR-AcIE0, CfEcR-OpIE1 and CfEcR-OpIE0 (Fig. 1B). Previous studies showed that translation of AcMNPV and OpMNPV ie0 produces both IE1 and IE0 due to internal initiation of translation within the AAD (Theilmann et al., 2001). However, ie0 constructs that have point mutations in the internal ATGs were shown to produce only IE0. Therefore, to ensure that chimeric CfEcR proteins were produced that only contained the IE0 AAD, two additional constructs were made that had mutations in the IE0 AAD. CfEcR-AcIE0Δ and CfEcR-OpIE0Δ were made containing IE0 AAD point mutations (Fig. 1B). In CfEcR-AcIE0Δ, the methionine ATG codon at 163 bp downstream of the first ATG start codon was changed to GCG (Fig. 1B). In CfEcR-OpIE0Δ, the second and third ATG codons at 106 bp and 118 bp downstream of the first ATG start codon were changed to GGG (Fig. 1B). All constructs were compared with CfEcR-VP16, which contains the archetypal mammalian-derived AAD and has been previously described (Palli et al., 2003).

Comparison of AcMNPV AADs and OpMNPV AADs in insect cells

To assay the transcriptional activities of baculovirus AADs in insect cells, we used a reporter EcRE-CAT, which contains the CAT gene under the transcriptional control of seven copies of hsp27 ecysone response element (7 × EcRE) and the OpMNPV ie2 basal minimal promoter containing a TATA box and CAGT element (Fig. 1A). In the presence of inducer RG-102240, the chimeric CfEcR associates with endogenous ultraspiracle (USP) to form a functional heterodimer that binds the EcRE to activate CAT gene expression in insect cells.

Initial experiments were done to optimize the induction time by the inducer RG-102240, a synthetic analogue of ecysone, in Ld652Y insect cells that had been transfected with CfEcR-AcIE0 and the reporter EcRE-CAT. Both CAT expression levels and protein levels of the CfEcR chimeras were determined (Fig. 2). After 5 h induction with RG-102240, increased levels of CfEcR-AcIE0 were observed and then remained constant up to 48 h of induction. Two protein bands were observed in cells transfected with CfEcR-AcIE0 (Fig. 2A): the higher molecular mass band corresponded to the expected size of the full-length CfEcR-AcIE0. The lower molecular mass band agreed with the expected size of proteins being internally translated from the IE1 start codon, as has been previously observed with the native ie0 mRNA (Theilmann et al., 2001). Transactivation analysis demonstrated that CAT expression peaked by 21 h of induction and remained constant up to 37 h of induction (Fig. 2B). Induction for 43–48 h resulted in lower CAT expression levels from CfEcR-AcIE0.

These results showed that suitable protein expression levels and peak levels of transactivation were obtained after 21–37 h of induction with 0.5 µM RG-102240 in transfected insect cells. Therefore, in subsequent experiments gene expression was induced for 31 h with 0.5 µM RG-102240 and cells were harvested at 37 h post-induction. Initial comparison of all the CfEcR chimeric constructs was performed in Ld652Y cells co-transfected with the chimeric EcRs and reporter EcRE-CAT. Western blotting demonstrated that all chimeric CfEcR proteins were expressed with the expected protein size in the presence or absence of RG-102240 (Fig. 3A). CfEcR-AcIE0 and CfEcR-OpIE0 produced two bands. However, as expected, CfEcR-AcIE0Δ and CfEcR-OpIE0Δ gave single bands corresponding to chimeras containing only the IE0 AAD. These results indicated that the mutation of the internal ATGs of the AADs prevented internal translation initiation. Interestingly, we observed that the translation of CfEcR-AcIE0 in Ld652Y cells produced more IE0 AAD than the IE1 AAD-containing CfEcR chimera, whereas CfEcR-OpIE0 produced more IE1 AAD than the IE0 AAD-containing chimera. It is not known whether the ratio of these two forms would affect the activation potential of these constructs.
Comparison of baculovirus IE1 and IE0 AADs

In Ld652Y cells, in the presence of RG-102240, transactivation analysis showed that chimeric CfEcRs containing the AADs from OpMNPV IE1, IE0 and IE0Δ gave 40% lower transactivation levels than the chimeras containing the AcMNPV AADs or VP16 AAD. In the absence of the inducer RG-102240, an obvious background expression was observed in all chimeric samples. CfEcR-VP16 gave the highest background expression in the absence of RG-102240. In the presence of RG-102240, the reporter construct alone resulted in expression levels that were 20% of the CfEcR-VP16 level, indicating that endogenous cellular EcR and USP within insect cells were activating reporter gene expression.

To compare the relative ability of the chimeric proteins in Ld652Y cells, the levels of expression of the chimeric CfEcRs in the presence of GSE were densitometrically measured and normalized relative to the CAT activity rates. CfEcR-OpIE0Δ gave the highest level of activity relative to protein density for the baculovirus AADs. However, CfEcR-AcIE1 and CfEcR-AcIE0 containing the AcMNPV AADs were more active than the corresponding CfEcR-OpIE1 and CfEcR-OpIE0. Despite being expressed from identical constructs, the protein levels of CfEcRs containing the OpMNPV AADs were never as high as the AcMNPV AAD-containing constructs. Therefore, despite being more active on a per molecule basis, absolute levels of gene activation by the most active construct, CfEcR-IE0Δ, were never greater than the AcMNPV-containing constructs. It is not known why chimeric CfEcR constructs containing OpMNPV AADs were not expressed at equal levels to AcMNPV AAD constructs in Ld652Y cells.

In Sf9 cells, similar results were observed (Fig. 4). Western blot analysis showed that all chimeric proteins were correctly expressed in Sf9 cells in the presence or absence of RG-102240 (Fig. 4A). Similar to the results from Ld652Y cells, the translation of CfEcR-AcIE0 and CfEcR-OpIE0 produced two proteins, while CfEcR-AcIE0Δ and CfEcR-OpIE0Δ produced only single proteins (Fig. 4A). CfEcR-AcIE0 was translated at almost equal levels to IE1 AAD- and IE0 AAD-containing CfEcRs; however, CfEcR-OpIE0 produced more IE1 AAD than IE0 AAD-containing CfEcRs; however, CfEcR-OpIE0 produced more IE1 AAD than IE0 AAD-containing CfEcRs. In the absence of the inducer RG-102240, a background expression was detected in all cell samples and the reporter gene alone gave an induction level of 13% compared with the CfEcR-VP16 level (Fig. 4B).

The levels of protein expression of the chimeric CfEcRs in the presence of GSE were densitometrically measured and normalized relative to the CAT activity rates (Fig. 4C). CfEcR-AcIE0Δ and CfEcR-OpIE0Δ gave the highest levels of activities relative to protein density, while CfEcR-VP16,

**Fig. 3.** Comparison of transactivation levels from chimeric CfEcRs containing baculovirus and VP16 AADs in Ld652Y cells. Ld652Y cells were co-transfected with reporter EcRE-CAT and chimeric CfEcRs and were harvested at 48 h post-transfection after 31 h of RG-102240 induction. (A) Western blot analysis of the chimeric CfEcR proteins from transfected Ld652Y cells with (+) or without (−) RG-102240. CfR-specific proteins were detected using a mAb specific for EcR. (B) Relative CAT activity in Ld652Y cells from the reporter EcRE-CAT transactivated by chimeric CfEcRs with (+) or without (-) RG-102240. CAT activities were relative to CfEcR-VP16, which was set at 100%. The CAT data were from at least two separate co-transfections, each in duplicate. (C) Relative CAT activity relative to protein density in Ld652Y cells in the presence of RG-102240. The levels of protein expression of the chimeric CfEcRs were densitometrically measured and normalized relative to the CAT expression in (B).
levels of activation from the chimeric CfEcRs containing the OpIE0 gave similar levels (Fig. 4C). Therefore, the higher levels of activation from the chimeric CfEcRs containing the AcIE1, CfEcR-AcIE0, CfEcR-OpIE1 and CfEcR-OpIE0 gave similar levels (Fig. 4C). Therefore, the higher levels of activation from the chimeric CfEcRs containing the AcMNPV AADs are due to the higher levels of protein expression in comparison with OpMNPV AAD constructs.

Results from both Ld652Y and Sf9 cells demonstrated that the AADs from AcMNPV and OpMNPV IE1 and IE0 can form functional chimeric proteins with the C. fumiferana EcR and inducibly transactivate gene expression when partnered with the endogenous USP. The AcMNPV AADs appear to activate reporter gene expression by approximately 20–40 % more in absolute terms in both Ld652Y and Sf9 cells. In terms of specific activity, in Ld652Y cells the AcMNPV IE1 and IE0 domains appeared to be more potent than the corresponding OpMNPV domains but the OpMNPV IE0Δ AAD was the most potent. However, in Sf9 cells (Fig. 4C), the AcMNPV and OpMNPV IE1 and IE0 AADs were approximately equal in their ability to activate gene expression when fused to the CfEcR. The IE0Δ domain from both viruses had the highest specific activity, with CfEcR-OpIE0Δ being the highest. These results show that the potency of the AAD can vary depending on the cell type.

Functional analysis of baculovirus AADs in mammalian NIH-3T3 and CHO cells

The results described above demonstrate that the AADs of IE1 and IE0 from baculoviruses AcMNPV and OpMNPV can function in EcR-based inducible gene expression system in insect cells, similar to the mammalian VP16 AAD. It was not known whether the insect-derived baculovirus AADs would be functional in a heterologous EcR-based inducible gene expression system in mammalian cells. We therefore constructed a mammalian cell EcR-based inducible gene expression system (Fig. 5). This gene expression system consisted of: (i) a reporter GAL4RE-CAT containing the CAT gene under the transcriptional control of five copies of the GAL4 response elements (5 × GAL4RE) and the OpMNPV ie2 basal minimal promoter (Fig. 5A); (ii) chimeric MmRXR containing the various baculovirus AADs and the VP16 AAD (Fig. 5B); (iii) a heterodimerization partner construct G:CfE(DEF) containing the GAL4 DNA-binding domain and the D, E and F domains of CfEcR (Fig. 5C) (Palli et al., 2003). In the presence of inducer RG-102240, the chimeric MmRXR and G:CfE(DEF) associate to form a functional heterodimer that binds to GAL4RE to activate CAT gene expression.

The initial mammalian cell line tested was NIH-3T3 (Fig. 6). The polyclonal antibody against MmRXR was unable to detect the baculovirus AAD chimeric proteins. Therefore, to confirm expression in mammalian cells, two separate mAbs specific for the AADs of AcMNPV and OpMNPV IE1 were used. Western blots showed that chimeric MmRXR proteins containing the baculovirus AADs were expressed with the expected sizes (Fig. 6A). The translation of the chimeric MmRXR-AcIE0 produced two proteins, while the MmRXR-OpIE0 produced predominately only one protein, which corresponded to the size of MmRXR-OpIE0 (Fig. 6A).
suggested that recognition of the IE0 internal translation start site can vary depending on the cell type and domain origin.

Transactivation analysis showed that, in the presence of 10 μM RG-102240, MmRXR-AcIE1, MmRXR-AcIE0 and MmRXR-AcIE0Δ activated gene expression to 122 %, 118 % and 94 %, respectively, relative to the MmRXR-VP16 (Fig. 6B). This surprising result showed that the insect baculovirus-derived AADs can activate gene expression in mammalian cells as well as or better than the most potent mammalian-derived AAD. However, MmRXR-OpIE1, MmRXR-OpIE0 and MmRXR-OpIE0Δ showed very low levels of transactivation at 12 %, 15 % and 5 % of the activity of MmRXR-VP16, respectively (Fig. 6B). In the presence or absence of RG-102240, unlike insect cells, no CAT expression was observed above background levels in any experiments.

As indicated above, the chimeric proteins MmRXR-AcIE1, MmRXR-AcIE0 and MmRXR-AcIE0Δ exhibited activity that was as strong as or stronger than MmRXR-VP16 in NIH-3T3 cells. These constructs were further examined to determine whether induction time could affect the ability of these chimeric MmRXRs to activate transcription. As shown in Fig. 6(C), expression levels continued to increase up to 79 h induction. However, 55 h was required to achieve significant levels of gene expression from all constructs. This result clearly demonstrated that MmRXR-AcIE1 and MmRXR-AcIE0 continued to activate gene expression to higher levels than MmRXR-VP16. Thus, the AADs from AcMNPV IE1 and IE0 consistently exhibited transactivation levels that were the same as or stronger than VP16 AAD in mammalian NIH-3T3 cells.

The baculovirus AADs were functionally tested in a second mammalian CHO cell line. The results showed that, relative to MmRXR-VP16, the chimeric MmRXR containing the baculovirus AADs MmRXR-AcIE1, MmRXR-AcIE0, MmRXR-AcIE0Δ, MmRXR-OpIE1, MmRXR-OpIE0 and MmRXR-OpIE0Δ all gave moderate levels of inducible gene activity (48, 55, 45, 46, 75 and 25 %, respectively) (Fig. 7). In the absence of RG-102240, no background expression was observed and reporter plasmid alone did not exhibit any activity in the presence or absence of RG-102240 in any experiments. Interestingly, in CHO cells MmRXR-OpIE0Δ gave the highest level of gene activation among the baculovirus AAD-containing MmRXRs, demonstrating that the host cell can play a significant role in the ability of these AAD transactivating domains to function.

Collectively, these data from both NIH-3T3 and CHO cells demonstrate, for the first time, that the baculovirus AADs are functional in mammalian cells, although the cell type and viral origin of AAD appear to have a significant effect on the activation potential. These results also suggest that the AcMNPV AADs are more universal in their ability to activate gene expression in multiple cell types than the OpMNPV AADs, based on the comparison of NIH-3T3 and CHO cells.

**DISCUSSION**

In this study, we have compared the ability of AADs derived from AcMNPV and OpMNPV IE1 and IE0 to transactivate gene expression in two heterologous EcR-based inducible expression systems in both insect and mammalian cell lines. Our results show that: (i) baculovirus AADs are functional
for transcriptional activation in both mammalian and insect cells; (ii) in general the AcMNPV AADs are more potent than OpMNPV AADs in Ld652Y, Sf9 and NIH-3T3 cells; and (iii) the AADs from AcMNPV IE1 and IE0 consistently gave higher levels of activation in mammalian NIH-3T3 cells than the archetypal AAD from the herpesvirus VP16.

AcMNPV, the prototype baculovirus, has a broad host range both in vivo and in vitro, reportedly infecting at least 33 species of lepidopteran larvae in 10 families as well as more than 25 different insect cell lines (Ayres et al., 1994; Danyluk & Maruniak, 1987; Lynn & Hink, 1980). OpMNPV in comparison has a narrow host range, infecting only a few insect species (Ahrens et al., 1997). The results of this study
Comparison of baculovirus IE1 and IE0 AADs

comparing the key regulatory domain of the essential proteins IE1 and IE0 appear to parallel the phenotype of the viruses. It has been shown that AADs interact with PolIII, general transcription factors or associated factors (Carey & Smale, 1999). In Ld652Y cells, the chimeric CIEcRs containing the AcMNPV IE1 and IE0 AADs had higher activities than those containing the corresponding OpMNPV AADs (Fig. 3B). However, in S9 cells, the IE1 and IE0 constructs were approximately equal in their potency for gene activation. Overall, our results suggest that the AcMNPV IE1 AAD may be more promiscuous and interact more effectively with transcription factors in both insect and mammalian cells. Therefore, it is possible that the AcMNPV IE1 AAD may play a role in enabling this virus to have a broad host range. However, based on densitometric analysis, the OpMNPV IE0Δ domain was the most active but the protein expression levels were never high, resulting in lower absolute levels of gene activation. Therefore, other factors such as protein stability may also play a key role in determining the effectiveness of the individual domains; this remains to be determined.

The function of IE0 in baculovirus infections is still unclear. Our previous studies have shown that in the context of the native C-terminal domain, IE0 is a stronger activator of specific viral promoters than IE1 (Theilmann et al., 2004). However, it is not known whether the additional amino acids added to the N terminus affect the AAD directly or have alternative functions such as cofactor recruitment. The results show that when fused to the CIEcR or the MmRXR, IE0Δ AAD consistently gave equal or slightly lower levels of expression relative to IE1 for both AcMNPV and OpMNPV domains but absolute expression levels were consistently lower, as indicated above. Therefore, these results indicate that, when tethered to a heterologous promoter by either the EcR or GAL4 DNA binding domains, the IE0-specific domain does not significantly affect the AAD and the ability to transactivate the reporter gene in absolute levels but appears to increase the specific activity.

Significant differences between the AcMNPV and OpMNPV domains were observed in mammalian cells. In NIH-3T3 cells, AcMNPV-activated gene expression was consistently higher than AcMNPV activities with those containing the corresponding OpMNPV AADs (Fig. 3B). However, in S9 cells, the IE1 and IE0 constructs were approximately equal in their potency for gene activation. Overall, our results suggest that the AcMNPV IE1 AAD may be more promiscuous and interact more effectively with transcription factors in both insect and mammalian cells. Therefore, it is possible that the AcMNPV IE1 AAD may play a role in enabling this virus to have a broad host range. However, based on densitometric analysis, the OpMNPV IE0Δ domain was the most active but the protein expression levels were never high, resulting in lower absolute levels of gene activation. Therefore, other factors such as protein stability may also play a key role in determining the effectiveness of the individual domains; this remains to be determined.

The function of IE0 in baculovirus infections is still unclear. Our previous studies have shown that in the context of the native C-terminal domain, IE0 is a stronger activator of specific viral promoters than IE1 (Theilmann et al., 2004). However, it is not known whether the additional amino acids added to the N terminus affect the AAD directly or have alternative functions such as cofactor recruitment. The results show that when fused to the CIEcR or the MmRXR, IE0Δ AAD consistently gave equal or slightly lower levels of expression relative to IE1 for both AcMNPV and OpMNPV domains but absolute expression levels were consistently lower, as indicated above. Therefore, these results indicate that, when tethered to a heterologous promoter by either the EcR or GAL4 DNA binding domains, the IE0-specific domain does not significantly affect the AAD and the ability to transactivate the reporter gene in absolute levels but appears to increase the specific activity.

Significant differences between the AcMNPV and OpMNPV domains were observed in mammalian cells. In NIH-3T3 cells, AcMNPV-activated gene expression was consistently better than VP16, whereas the OpMNPV domains barely activated gene expression. However, in CHO cells, OpMNPV AADs were equal to or better than AcMNPV AADs. These results highlight the cell dependence of AAD gene activation and the promiscuous nature of the AcMNPV IE1 AAD. Previous studies have shown that the native AcMNPV IE1 is able to activate gene expression in mammalian BHK-21 cells (Carbonell et al., 1985; Carbonell & Miller, 1987; Murges et al., 1997). Our results indicate that this is due, at least in part, to the AAD domain of IE1, which appears to be a more universal activation domain, similar to VP16 AAD. However, even though the AcMNPV IE1 AAD was more active in NIH-3T3 cells, the VP16 domain had consistently strong transactivation in all cell types, unlike the baculovirus domains, which were quite variable depending on cell type.

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