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Defining the roles of the baculovirus regulatory proteins IE0 and IE1 in genome replication and early gene transactivation

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ABSTRACT

IE0 and IE1 of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus are essential transregulatory proteins required for both viral DNA replication and transcriptional transactivation. IE0 is identical to IE1 except for 54 amino acids at the N-terminus but the functional differences between these two proteins remain unclear. The purpose of this study was to determine the separate roles of these critical proteins in the virus life cycle. Unlike prior studies, IE0 and IE1 were analyzed using viruses that expressed *ie0* and *ie1* from an identical promoter so that the timing and levels of expression were comparable. IE0 and IE1 were found to equally support viral DNA replication and budded virus (BV) production. However, specific viral promoters were selectively transactivated by IE0 relative to IE1 but only when expressed at low levels. These results indicate that IE0 preferentially transactivates specific viral genes at very early times post-infection enabling accelerated replication and BV production.

Introduction

The immediate early proteins IE0 and IE1 are the key transregulatory proteins in the alphabaculovirus replication cycle. In the type species of the alphabaculoviruses, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), both IE0 and IE1 have been shown to transcriptionally regulate early and late genes (Choi and Guarino, 1995a; Choi and Guarino, 1995b; Choi and Guarino, 1995c; Guarino and Summers, 1986a; Huijskens et al., 2004; Kovacs et al., 1991; Kremer and Knebel-Morsdorf, 1998; Nissen and Friesen, 1989; Olson et al., 2001; Olson et al., 2002; Passarelli and Miller, 1993) as well as function as replication factors (Kool et al., 1994a; Luria et al., 2012; Pathakamuri and Theilmann, 2002; Stewart et al., 2005; Taggart et al., 2012). IE0 and IE1 are translated from distinct *ie0* and *ie1* mRNA transcripts which arise from spliced or unspliced mRNAs generated from two discrete promoters of the *ie0–ie1* gene complex. Additional spliced viral RNAs have recently been identified (Chen et al., 2013) but the *ie0–ie1* gene complex is still the only known spliced gene within the baculovirus genome that is processed into multiple protein products (Chisholm and Henner, 1988; Theilmann et al., 2001). The splicing event results in IE0 being identical to IE1 except for an additional 54 amino acids of no known function at its N-terminus (Chisholm and Henner, 1988). The presence of either IE0 or IE1 is essential for viral replication but both proteins are required for a wildtype infection (Stewart et al., 2005). Both IE0 and IE1 are present throughout the AcMNPV replication cycle, although their levels of expression are not equal. IE0 expression and abundance peaks during the first few hours post-infection prior to the initiation of DNA replication and declines thereafter (Huijskens et al., 2004). IE1 becomes more abundant than IE0 by the time replication begins and continues to increase throughout infection (Chen et al., 2013; Choi and Guarino, 1995b; Huijskens et al., 2004; Theilmann and Stewart, 1991). One of the most surprising characteristics of the spliced *ie0* transcript is that it is translated as both IE0 and IE1 due to internal translation initiation at the *ie1* start codon resulting in both proteins always being present (Theilmann et al., 2001). The reason that alphabaculoviruses

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produce IE0 in addition to IE1 is not clear, although the conservation of IE0 in the alphabaculoviruses suggests that this form of the protein serves an important or unique role. Both proteins appear to have similar transregulatory roles but no unique function has been identified for either protein. However, because both proteins must be present to achieve wildtype levels and progression of infection, it is important to understand the functional roles of both proteins, to fully understand baculovirus pathology.

IE0 or IE1 can transactivate genes in an enhancer-dependent or independent manner (Nissen and Friesen, 1989; Rodems and Friesen, 1993; Theilmann and Stewart, 1991). However, to date, the only DNA element that IE0 and IE1 have been shown to bind to are *hr* elements (Choi and Guarino, 1995a; Olson et al., 2003), which act as enhancers of transcription (Guarino and Summers, 1986b) as well as origins of replication (Leisy and Rohrmann, 1993; Pearson et al., 1992). In the absence of enhancer elements, IE0 and IE1 transactivate many early genes in transient assays but no specific IE0 or IE1 responsive element within the early gene promoters has been identified. This suggests that in the absence of enhancers, IE0 or IE1 may activate transcription by an indirect mechanism and not bind to the promoter directly. IE0 and IE1 form IE0-IE0 and IE1-E1 homodimers and IE0-IE1 heterodimers and all three dimer forms bind to enhancer elements (Kremer and Knebel-Morsdorf, 1998; Olson et al., 2001). In all prior comparisons of IE0 and IE1 transactivation, the two proteins were expressed under the control of their native promoters, resulting in a different temporal pattern of expression and differing levels of expression. It is unknown whether IE0 and IE1 transactivate promoters with the same efficiency.

IE0 or IE1 is also required for AcMNPV DNA replication, in conjunction with the replication factors LEF1, LEF2, LEF3, LEF11, viral DNA polymerase (DNApol) and helicase. Viral DNA replication is also augmented by the non-essential factors P35, LEF7, IE2 and PE38 (Kool et al., 1994a, 1995, 1994b; Lin and Blissard, 2002; Luria et al., 2012). In infected cells IE1 binds to *hr* elements and co-localizes in nuclear structures, thought to be viral replication factories (Kawasaki et al., 2004; Nagamine et al., 2006; Okano et al., 1999). Within these structures IE0 and IE1 may be acting as origin binding proteins allowing the replisome complex to form due to binding to *hr* elements (Blissard and Rohrmann, 1991; Choi and Guarino, 1995a; Lu and Carstens, 1993; Mikhailov, 2003; Rodems et al., 1997).

One of the functional domains within IE0 and IE1 is an N-terminal transcriptional acidic activation domain (AAD) which also contains a domain essential for viral DNA replication (Pathakamuri and Theilmann, 2002; Taggart et al., 2012). The replication domain contains a motif that resembles a cyclin-dependent phosphorylation site (TPXR/H) and amino acid substitution in this region caused loss of DNA replication activity (Taggart et al., 2012). Interestingly, the ability to support viral DNA replication is not maintained when the *Orgyia pseudotsugata* MNPV IE1 AAD is replaced with the heterologous AcMNPV AAD, indicating that this region contributes to the specificity of the virus DNA replication complex (Pathakamuri and Theilmann, 2002). If the replication domain is inactivated, IE1 can remain functional for transcriptional transactivation (Pathakamuri and Theilmann, 2002; Taggart et al., 2012). This indicates that transcriptional transactivation functions and viral DNA replication functions of IE1 are independent.

Past studies have shown that IE0 and IE1 both support viral DNA replication, but appear to play different roles. Recombinant viruses expressing only IE0 show a delay in onset of DNA replication compared to wildtype, but viral DNA accumulates to higher levels at later times post-infection. Whereas viruses expressing only IE1 initiate DNA replication and attain levels similar to wildtype virus (Stewart et al., 2005). In these experiments however, IE0 and IE1 were expressed under the control of their native promoters resulting in different temporal kinetics and expression levels of each protein. The impact observed on viral DNA replication could therefore be simply due to quantitative expression differences potentially masking any functional differences between the two proteins.

In this study, to determine the functional differences between IE0 and IE1 they were analyzed and compared by expressing both genes under control of the same promoter. This approach permitted similar temporal expression and similar levels of protein at very early times of post-infection. Results showed that IE0 and IE1 equally supported BV production and DNA replication in virus infected cells. However, significant differences were observed in transient transactivation studies which showed for the first time that IE0 preferentially transactivates a subset of viral early gene promoters.

Results

Construction of ie0 and ie1 knockout viruses and repair viruses

To investigate the function of IE0 and IE1, a *ie0–ie1* knockout virus (AcBac$^{ac146\text{-}ie1KO}$) was made to serve as a backbone for the construction of viruses expressing *ie0*, *ie0*^{MtoA} or *ie1* under control of the *gp64* promoter to achieve similar levels of IE0 and IE1 expression. The knockout deleted the entire *ie1* ORF, which also results in the deletion of *ie0*. The deletion of *ie1* ORF also deletes the promoter of the essential gene *ac146* (Dickison et al., 2012) which is contained within the *ie1* ORF (Fig. 1A). To account for this overlap, the complete ORFs of both *ac146* and *ie1* were deleted by replacement of *ac146-ie1* with an EM7-promoter-*zeocin* cassette by homologous recombination and the *ac146* ORF was reinserted into all repair viruses (Fig. 1A).

To compare the function of IE0 and IE1, the *ie0–ie1* knockout bacmid, AcBac$^{ac146\text{-}ie1KO}$, was repaired with a series of constructs containing either *ie0*, *ie0*^{MtoA} or *ie1* under control of the *gp64* promoter generating the viruses, v*gp64p*-IE1 which only produces IE1, v*gp64p*-IE0 which produces both IE0 and IE1, and v*gp64p*-IE0^{MtoA} which only produces IE0 because the IE1 initiation codon Met was changed to Ala (Fig. 1A). The *gp64* promoter was chosen because it is an immediate-early promoter that would permit the same temporal expression of IE0 and IE1 as it is constitutively active in insect cells (Blissard and Rohrmann, 1991; Chisholm and Henner, 1988; Guarino and Summers, 1988) and the AcMNPV promoter has been shown to be unaffected by IE0 or IE1 except for basal level transactivation (Nie, 2010). The repair viruses also inserted the *polyhedrin* (*polh*) gene and the *green fluorescence protein* (*gfp*) marker protein gene (Fig. 1A). The viral bacmids were transfected into *Spodoptera frugiperda clone 9 (Sf9)* cells to confirm virus viability and to confirm correct expression of IE0 and IE1 by Western blot. BV stocks were generated from bacmid transfected cells, titred and used for further experiments.

Expression of IE0 and IE1 under control of the gp64 promoter

To compare the temporal expression of IE0 and IE1 a time course of infection was performed and the expression of IE0 and IE1 was analyzed from each of the viruses plus wildtype (Fig. 1B). Expression of both IE1 and IE0 from v*gp64p*-IE1 and v*gp64p*-IE0^{MtoA} followed the same temporal pattern that is steadily increasing in expression levels up to 48 hpi with the largest increase between 24 and 36 hpi. Expression of IE0 and IE1 from v*gp64p*-IE0 differed from the other two viruses in that it had a more rapid increase in levels starting between 12 and 24 hpi. During the early period prior to, and concomitant with initiation of