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Functional Dissection of the *Autographa californica* Nuclear Polyhedrosis Virus Immediate-Early 1 Transcriptional Regulatory Protein

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***Autographa californica* multicapsid nuclear polyhedrosis virus-infected insect cells express a viral immediate-early transcriptional regulatory protein, IE1, that has been shown by transient-expression assays to stimulate the expression of certain baculovirus delayed-early (DE) promoters and to inhibit the expression of other immediate-early (IE) genes. It is believed that certain DE promoters are activated, in part, by direct interactions between IE1 and enhancer elements located in regions adjacent to these genes. We have used transient cotransfection and DNA-binding assays to examine the function of mutant forms of IE1. Our results indirectly show that IE1 has at least two separable domains that are essential for its role in the modulation of baculovirus gene expression. A domain rich in acidic residues and essential for transactivation is located within the N-terminal 145 amino acids of the polypeptide. A second domain, located in the C-terminal 437 amino acids of IE1, is required for inhibitory and DNA-binding activities. Several nontransactivating IE1 mutants *trans*-dominantly interfered with wild-type IE1 transactivation of enhancer-linked DE genes. *trans*-dominant interference was expressed only by IE1 mutants that retained the N-terminal putative acidic activation domain, suggesting that this region may be involved in associations with a factor(s) essential for activation of enhancer-linked genes.**

Viruses have served as model systems for studies of gene expression and regulation in both prokaryotic and eukaryotic cellular systems. Viral gene expression is tightly regulated by interactions between viral and host cell factors. Most viruses express their genes in temporally ordered tiers that can be classified according to their kinetics of expression, dependence on viral DNA replication, and requirement for the prior expression of one or more viral regulatory factors. Baculovirus genes have been grouped into three temporal classes: early (E), late (L), and very late (VL; for a review, see reference 1). E genes are distinguished from L and VL genes in that their expression is independent of viral DNA replication. Additionally, E genes are transcribed by an α-amanitin-sensitive RNA polymerase II activity, whereas L and VL transcription is controlled by a virus-encoded/regulated α-amanitin-insensitive activity (22). E genes have been further divided into two subgroups (immediate-early [IE] genes and delayed-early [DE] genes) on the basis of transient-expression (3, 16, 29) and cycloheximide blockage (25, 35) experiments. IE promoters are fully active in the absence of other viral factors. DE promoters require one or more IE gene products for full activation (3, 10, 16, 25, 35, 36), although some low-level expression may be detected in the absence of viral transactivators (41). From several studies, it is believed that IE gene products function as viral gene regulators (3–5, 16, 18, 29, 30, 41) and that some DE factors control viral DNA replication (32, 37, 42). L and VL genes encode structural proteins involved in viral encapsidation and polyhedron assembly. In sum, the entire baculovirus life cycle is believed to be initiated, in part, by IE gene products; a knowledge of the how these gene products function is central to the understanding of the biology of baculoviruses and the processes involved in the regulation of insect genes.

The IE1 gene of *Autographa californica* multicapsid nuclear polyhedrosis virus (Ac*M*NPV) has been shown, on the basis of transient-expression experiments, to encode a multifunctional regulatory protein (4, 17, 29). IE1 is transcribed from a single promoter throughout infection as an unspliced 1.9-kb RNA with an open reading frame (ORF) encoding a 70-kDa protein. A slightly longer form of IE1 (spliced-IE1 or IE0 [6]) is transcribed early and late from two different start sites located approximately 4 kbp upstream of the IE1 promoter (28). Studies have shown that IE1 induces the expression of several chimeric genes linked to DE (16, 17) and L (19) gene promoters, an activity termed enhancer independent. Two independent studies have also demonstrated that the IE1 gene product of both Ac*M*NPV (29) and *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus (Op*M*NPV [41]) has autoregulatory properties; i.e., it stimulates its own expression. IE1 not only transactivates promoters but also increases the transcription rate of several baculovirus and heterologous promoters *cis*-linked to homologous region (*hr*) enhancers, an activity termed enhancer dependent (17). Insect cells transfected with IE1 contain an enhancer-specific DNA-binding activity (14), leading us to believe that enhancer-dependent transactivation may be mediated by a direct interaction between the IE1 gene product and the viral enhancers. Lastly, IE1 not only stimulates gene expression but also inhibits the expression of chimeric genes linked to the IE0 (29) and IEN (4) promoters.

On the basis of its demonstrated activities in both transient-expression and DNA-binding assays, it was reasonable for us to hypothesize that the IE1 gene product, like other *trans*-acting factors, may contain genetically separable au-

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tonomous domains. Therefore, we constructed a number of N- and C-terminal IE1 deletion mutants and analyzed them by transient-expression and DNA-binding assays. Our results demonstrate that IE1 is a complex protein with at least two distinct regions: an N-terminal putative acidic activation domain, and a C-terminal region that is required for inhibition of IE0 and DNA binding. Our results also show that several nontransactivating forms of IE1 abrogate the ability of wild-type IE1 to transactivate an *hr*-linked gene; i.e., they act as *trans*-dominant repressors.

MATERIALS AND METHODS

Cell culture. *Spodoptera frugiperda* (Sf9) cells were passaged in spinner flasks at 27°C as previously described (40).

Plasmid constructions. A schematic representation of all IE1 plasmids used in this study is shown in Fig. 1A. All recombinant plasmids were constructed in vitro by standard cloning techniques (34) and sequenced (39). The wild-type IE1 plasmid (pNheIE1) was derived from pIE1 (16) by digestion with *Nhe*I and *Sma*I, repair of the 5′ extensions with the large fragment of DNA polymerase I, and subsequent religation. IE1 C-terminal deletion mutants were constructed by inserting a synthetic deoxyoligonucleotide linker (pCTAGCTAGCTAG), encoding nonsense codons in all three reading frames, into convenient restriction sites within the IE1 ORF. N-terminal mutants were derived from an IE1-encoding plasmid (29) that contains a unique *Bam*HI restriction endonuclease site immediately 3′ to the translation initiation codon (**ATG**GATCC; ATG in bold, *Bam*HI site underlined). Subsequently, this plasmid was digested with *Bam*HI and either *Ava*I, *Eco*RV, or *Dra*III to yield the mutants Δ2–16, Δ2–52, and Δ2–145, respectively. 5′ extensions derived from DNAs digested with *Bam*HI and either *Ava*I or *Eco*RV were repaired with Klenow polymerase, and a synthetic 10-mer *Bgl*II linker (pGAAGATCTTC) was inserted to maintain the proper reading frame. DNA digested with *Bam*HI and *Dra*III was blunt ended with T4 polymerase and subsequently religated.

The IE0/IE1 reporter (pGCAT) used in this study has been described previously (29). Briefly, it contains the chloramphenicol acetyltransferase (CAT) gene cloned 36 bp downstream of the IE1 ATG in pH3G (*Hin*dIII G fragment of Ac*M*NPV; 90.6 to 96.8 map units), and expresses IE0CAT and IE1CAT fused gene products. The IE1 nonsense mutant (pIE1ΔORF [29]), the DE 39-kDa reporters (39K reporters; p39CAT and p39CATQ− [17]), and the plasmid IBI-24 (14) containing the *hr5* enhancer have been previously described. The plasmid GC123 contains the IE0 ORF (derived from its cDNA) cloned under the control of its native promoter; it was obtained from Dennis Henner and George Chisholm.

CAT assays. Transfections were done by the calcium phosphate precipitation method (16). Briefly, 10^6 Sf9 cells were seeded onto six-well plates and allowed to attach for 1 h. Subsequently, the cells were transfected with plasmids and pUC DNA as carrier for a total DNA concentration of 10 μg/10^6 cells. Lysates were prepared 24 h posttransfection and assayed for CAT expression (12).

Radioimmunoprecipitation assays. Sf9 cells were seeded at a density of 10^6 cells per well in six-well plates and transfected with 10 μg of DNA per well. Approximately 15 h posttransfection, cells were incubated for 2 h in methionine-free Grace's medium. Subsequently, cells were radiolabeled with 100 μCi of [^{35}S]methionine (Amersham) per ml in methionine-free Grace's medium for 1 h at 27°C. Extracts were prepared (23) and incubated with either polyclonal anti-IE1 antiserum at a dilution of 1:500 or polyclonal anti-CAT antiserum (5′-3′) at a 1:250 dilution. Immunoprecipitates were collected (26) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (31) and autoradiography.

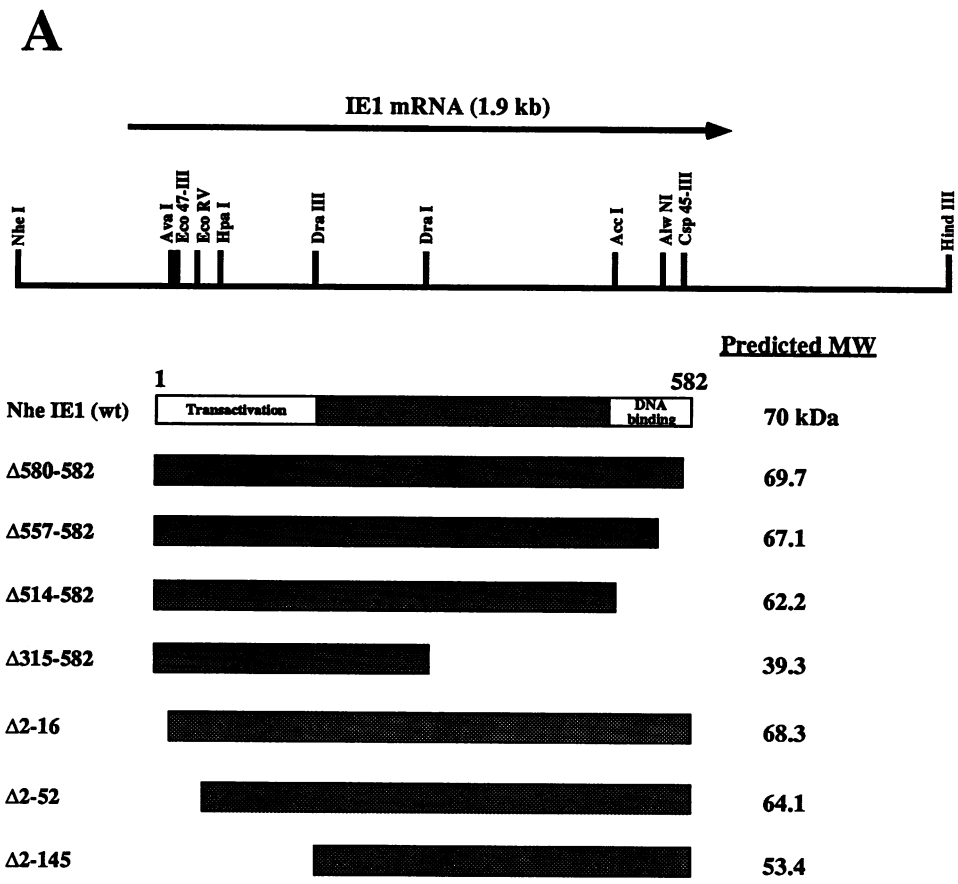
Electrophoretic mobility shift assay (EMSA). Sf9 cells were seeded at a density of 9 × 10^6 cells per T-75 flask and transfected with 90 μg of DNA. Approximately 24 h posttransfection, whole-cell extracts were prepared as described previously (14). A 252-bp fragment containing a portion of *hr5* was derived from pIBI-24 and end labeled with T4 polynucleotide kinase essentially as described previously (14). Protein-DNA complexes were separated by native gel electrophoresis and analyzed by autoradiography.

RESULTS

Analysis of the effect of IE1 deletions on DE gene enhancer-independent and -dependent transactivation. To investigate the structural elements essential for its activities, we conducted a functional dissection of the IE1 polypeptide. A series of N- and C-terminal deletion mutants (Fig. 1A) were constructed and subsequently tested by transient-expression assays. The wild-type plasmid (pNheIE1) used to construct deletion mutants contains the entire IE1 ORF, 428 bp of upstream regulatory sequences, and 640 bp of downstream sequences. This plasmid does not contain any IE0 regulatory or coding sequences; thus, Sf9 cells transfected with this plasmid express only unspliced IE1 RNA. Mutants were sequenced to determine deletion end points (Fig. 1B). Some C-terminal mutants have additional non-IE1-encoded amino acids at their C termini as a result of the synthetic linker used to introduce a translation termination signal. N-terminal mutants retain the native IE1 ATG translation initiation codon followed by sequences encoded by synthetic oligonucleotides inserted to maintain the proper reading frame.

A transient-expression assay was used to analyze the transactivation activities of IE1 deletion mutants. In these assays, wild-type or mutant IE1 and either p39CAT (enhancerless) or p39CATQ− (*hr5* enhancer-plus) were cotransfected into Sf9 cells. One day later, cell extracts were prepared and assayed for CAT activity. The cumulative results of this assay show that both enhancer-independent (Fig. 2A) and enhancer-dependent (Fig. 2B) 39K reporter transactivation required the same regions of the IE1 polypeptide. N-terminal deletion mutants lacking 15 (Δ2–16) or 51 (Δ2–52) amino acids transactivated gene expression, whereas the mutant lacking 144 (Δ2–145) residues did not. Cells transfected with pΔ2–16 consistently expressed 1.3- to 1.5-fold greater CAT activity than wild-type IE1-transfected cells did, whereas pΔ2–52-transfected cells expressed 0.5- to 0.75-fold less activity. The results of assays conducted with C-terminal mutants show that although deletion of 2 amino acids (Δ580–582) had no effect, deletions of 25 (Δ557–582), 68 (Δ514–582), or 267 (Δ315–582) residues ablated transactivation function.

To determine whether loss of transactivation was either caused by deletion of a functional domain(s) or by suboptimal expression and/or an inherent instability of the mutant gene products, we analyzed IE1 proteins in transfected Sf9 cells. For this experiment, a polyclonal rabbit antiserum raised against a bacterially expressed IE1 protein (7) was used in a radioimmunoprecipitation assay (Fig. 3). This antiserum specifically immunoprecipitated a protein of the expected molecular mass (70 kDa) of IE1 in transfected cells (Fig. 3, lane 2). This assay confirmed that, with the excep-



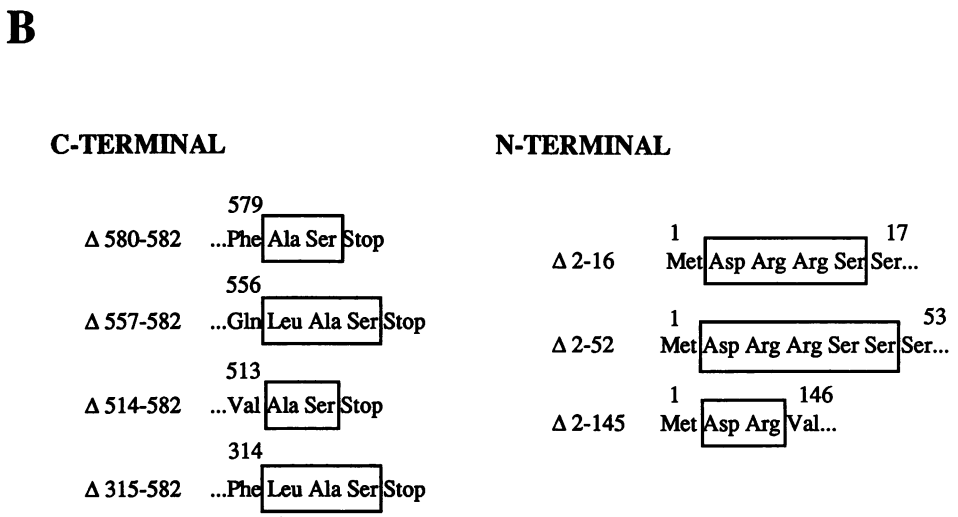


FIG. 1. Schematic representation of IE1 deletion mutants. (A) A partial restriction map denoting the restriction sites used in the construction of IE1 mutants is illustrated at the top. The arrow above the map denotes the size and transcriptional direction of the IE1 mRNA. The shaded rectangles represent IE1 coding sequences. The open rectangles denote putative functional domains. The predicted molecular masses (MW) of the deletion mutant polypeptides are shown to the right. (B) Predicted amino acid sequences of IE1 mutants at the deletion end points. These data were derived from DNA sequence analysis. The sequences encoded by oligonucleotides inserted into the IE1 reading frame are boxed. The numbers above the sequences denote IE1 amino acids.

tion of Δ2–145, the various active and inactive IE1 mutants were expressed at approximately equivalent levels. In Fig. 2 we showed that Δ2–145 did not transactivate the 39K reporter plasmids to any measurable extent. Since we were not able to detect this polypeptide, we could not attribute its lack of transactivation activity to the deletion of a functional domain.

Analysis of the autoregulatory and inhibitory activities of

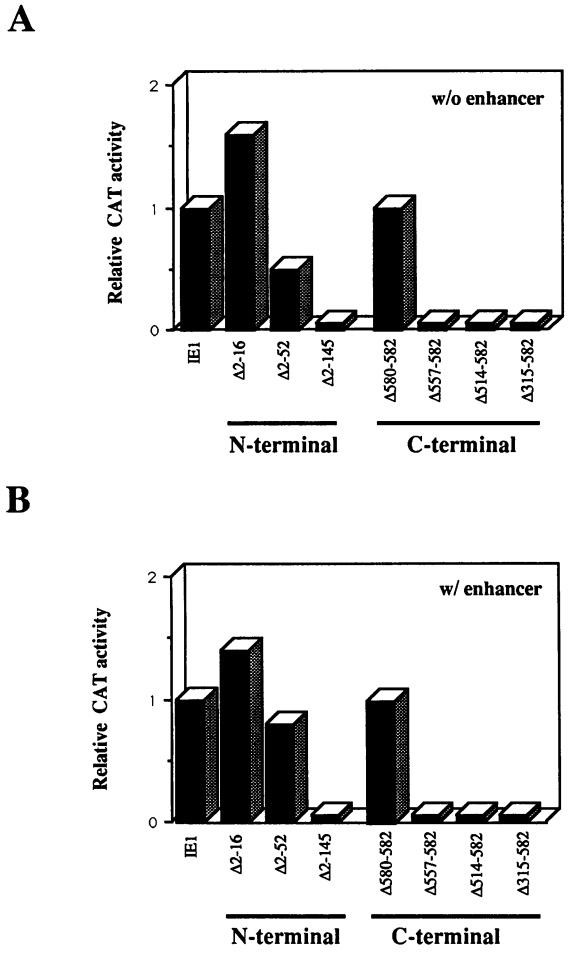


FIG. 2. Transactivation of 39K promoter-driven expression of CAT by IE1 deletion mutants. We transfected 10^6 Sf9 cells with 1 μg of p39CAT (without *hr5*) (A) or p39CATQ− (with *hr5*) (B) and 5 μg of the indicated IE1-expressing plasmids. Extracts were prepared 24 h posttransfection and analyzed for CAT activity (39CAT extracts were undiluted, whereas 39CATQ− extracts were diluted 1:1,000). The averages of three separate experiments are plotted as the level of transactivation relative to wild-type IE1.

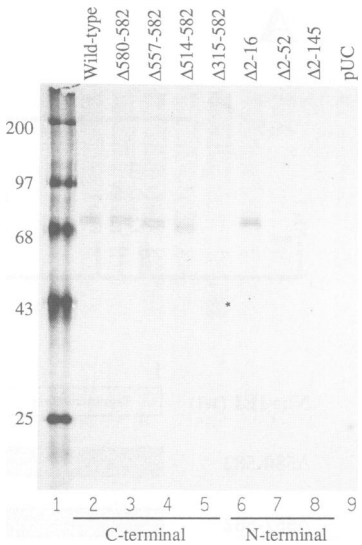


FIG. 3. Radioimmunoprecipitation of IE1 deletion mutant gene products. We transfected 3×10^6 Sf9 cells with 30 μg of the indicated IE1 plasmids. Cells were radiolabeled, and extracts were prepared and subsequently immunoprecipitated with rabbit polyclonal anti-IE1 antiserum. Immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The sizes of molecular mass standards (in kilodaltons) are shown to the left. The asterisk denotes the position of the Δ315–582 polypeptide which was expressed at a lower level than other IE1 proteins.

IE1 deletion mutants. As mentioned above, it has been shown that IE1 stimulates expression from its own promoter (29, 41), an activity we term autoregulation, and inhibits expression from the IE0 promoter (41). The experiment whose results are shown in Fig. 4 was done to determine whether the autoregulatory and inhibitory properties of IE1 could be genetically separated. A reporter plasmid (GCAT; Fig. 4B) that expresses fused CAT gene products under the control of both the IE0 and IE1 promoters was transfected either alone or in the presence of wild-type or mutant IE1 plasmids. We have previously shown that cotransfection of pGCAT with pNheIE1 decreases IE0CAT expression approximately 5-fold and increases IE1CAT expression 5- to 10-fold (29) (Fig. 4, compare lanes 2 and 4). In addition, we showed that an IE1 nonsense mutant (IE1ΔORF) has no effect on IE1CAT or IE0CAT expression (Fig. 4, compare lanes 2 and 3).

Cotransfection with mutant IE1 plasmids showed that deletions of 15 or 51 residues from the N terminus did not affect either autoregulatory or inhibitory activities (Fig. 4, lanes 5 and 6, respectively). In Fig. 2 we noted that Δ2–145 did not transactivate the delayed-early 39K reporter plasmids. In this experiment, Δ2–145 also failed to transactivate IE1CAT expression. However, it did inhibit IE0CAT expression to the same extent that wild-type IE1 did (Fig. 4, compare lanes 4 and 7), suggesting that the inhibitory and transactivation domains of IE1 may be separable. The fact that Δ2–145 inhibited IE0 expression also suggests that the lack of transactivation (Fig. 2) by this mutant is probably a result of the loss of an activation domain and not the result of poor expression of the mutant gene product. Lastly, the absence of autoregulatory and inhibitory activities in mutants deleted for more than 25 C-terminal residues (Fig. 4, lanes 9, 10, and 11) suggests that this region may be essential for these two activities.

Analysis of the DNA-binding properties of IE1 deletion mutants. Recent evidence from our laboratories has suggested that IE1 may be an enhancer-binding protein (14). The results of our functional assays with the deletion mutants prompted us to determine whether the loss of transactivation or inhibitory activities may result from the loss of a DNA-binding domain. An EMSA was conducted to determine which region(s) within IE1 is essential for DNA-binding activity. Sf9 cells were transfected with either wild-type or mutant IE1 plasmids, and 1 day later extracts were prepared and assayed by an EMSA with a double-stranded DNA probe derived from a plasmid which contains the *hr5* enhancer element. Extracts prepared from cells transfected with pUC DNA (Fig. 5, lane 9) contained a nonspecific binding activity that was not affected by the addition of excess unlabeled probe (data not shown). Wild-type IE1 and pΔ580–582-transfected cells contained an *hr5*-specific DNA-binding activity that resulted in the formation of at least two complexes (lanes 1 and 2, respectively). Deletions of 25 or

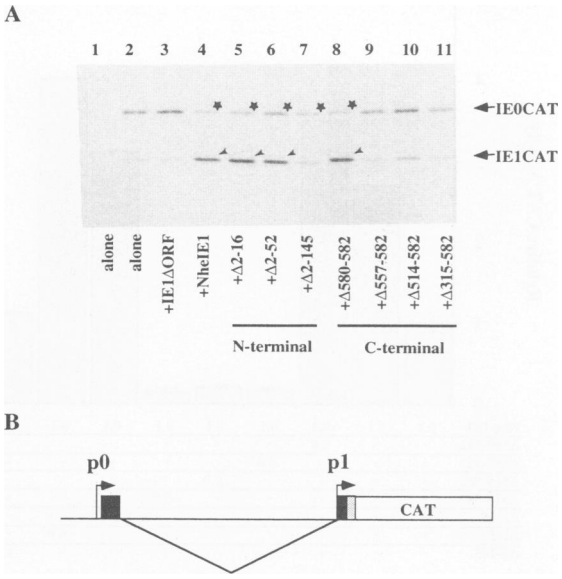


FIG. 4. Regulation of IE1 and IE0 promoter-driven expression of CAT by IE1 deletion mutants. (A) We cotransfected 10^6 Sf9 cells with 1 μg of pGCAT and 5 μg of the indicated IE1 plasmids. Cells were radiolabeled at 24 h postinfection for 2 h with [^{35}S]Translabel. Subsequently, detergent-soluble extracts were immunoprecipitated with rabbit anti-CAT antiserum. Immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The locations of IE1CAT and IE0CAT fusion proteins are shown on the right. Asterisks denote repressed expression of IE0CAT, and arrows indicate stimulation of IE1CAT expression. Lanes: 1, preimmune serum; 2 to 11, polyclonal rabbit anti-CAT serum. IE1ΔORF is a nonsense mutant of IE1. (B) Illustration of the reporter plasmid pGCAT. Arrows above the map denote the IE0 (p0) and IE1 (p1) transcription initiation sites. Intronic sequences are denoted below the map. Boxes denote coding regions: black, IE0; stippled, IE1; open, CAT. Not drawn to scale.

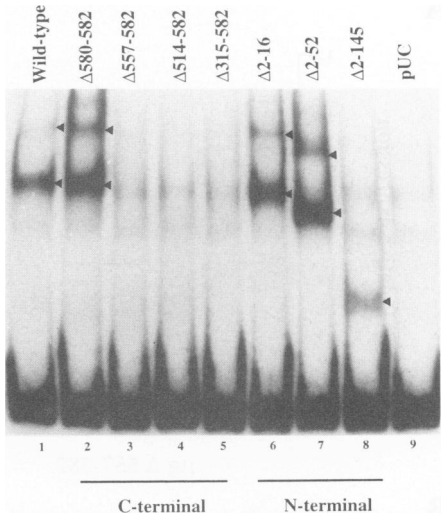


FIG. 5. DNA-binding activity of IE1 deletion mutants. We transfected 9×10^6 Sf9 cells with 45 μg of either wild-type IE1, mutant IE1, or pUC DNA. Binding lysates were prepared 24 h posttransfection and incubated with a radiolabeled *hr5* double-stranded DNA probe. Binding reactions were carried out at 4°C for 20 min, and the mixtures were subsequently analyzed by native PAGE. Arrowheads indicate specific protein-DNA complexes.

more C-terminal amino acids resulted in complete loss of binding activity (lanes 3, 4, and 5). Deletions from the N terminus resulted in sequentially faster-migrating complexes, providing evidence that IE1 is a component of the shifted complexes.

Analysis of *trans*-dominant interfering mutants of IE1. Many transcriptional regulatory proteins contain genetically separable domains that, when expressed as independent truncated gene products, sometimes interfere with the activity of the wild-type protein; i.e., they function as *trans*-dominant repressors (13, 20, 33, 43). Of the seven IE1 mutants, four did not transactivate gene expression. To further investigate the mechanism of IE1-mediated transactivation, we first tested Δ557–582 (a mutant that has neither transactivating nor DNA-binding activities) for the ability to interfere with wild-type IE1-mediated transactivation of DE gene expression. Sf9 cells were cotransfected with either p39CAT or p39CATQ−, a fixed amount of pNheIE1, and different amounts of pΔ557–582. Lysates were assayed for CAT expression 1 day later. The results of this experiment showed two very different effects on IE1-mediated transactivation (Fig. 6). The expression of p39CATQ− was decreased up to 10-fold by increasing the amount of pΔ557–582 (Fig. 6A); however, expression of p39CAT remained virtually unchanged (Fig. 6B). These data suggest that Δ557–582 may function as a *trans*-dominant repressor of IE1 enhancer-dependent transactivation. A control experiment with a nonsense mutant of IE1 (pIE1ΔORF) (29) in place of Δ557–582 showed that it had no effect on CAT expression from either reporter plasmid (data not shown), supporting the hypothesis that the inhibitory effect is mediated by the mutant IE1 gene product. These data do not rule out the possibility that the Δ557–582 gene product indirectly lowered 39CATQ− expression by inhibiting pNheIE1 expression. However, three lines of evidence argue against this hypothesis. First, as shown in Fig. 4, Δ557–582 had no effect on IE1CAT expression in pGCAT-transfected cells (compare lanes 2 and 9). Second, expression of an IE1CAT reporter plasmid was not affected by pΔ557–582 in a cotransfection experiment (data not shown). Lastly, Δ557–582 had no effect on p39CAT expression (Fig. 6B), suggesting that wild-type IE1 levels were unaffected.

These results strongly suggest that the Δ557–582 gene product directly interferes with the enhancer-dependent transactivation function of IE1. We tested the remainder of the IE1 deletion mutants in a similar assay in an attempt to isolate the region essential for interference activity. Since 5 μg of pΔ557–582 inhibited *hr*-linked CAT expression by approximately 90% (Fig. 6A), we transfected Sf9 cells with p39CATQ−, 0.1 μg of pNheIE1, and 5 μg of each IE1 mutant (Fig. 7). The results of this experiment demonstrate that all three nontransactivating C-terminal mutants were able to interfere with IE1 enhancer-dependent transactivation. In contrast to the other nontransactivating mutants, Δ2–145 failed to demonstrate *trans*-dominant interference. As expected, cells cotransfected with deletion mutants capable of transactivation expressed higher levels of CAT activity. Lastly, none of the nontransactivating mutants were able to interfere with the expression of p39CAT, suggesting that interference is enhancer dependent (data not shown).

A previous study in our laboratories demonstrated that IE0 has enhancer-dependent transactivation activity (29).

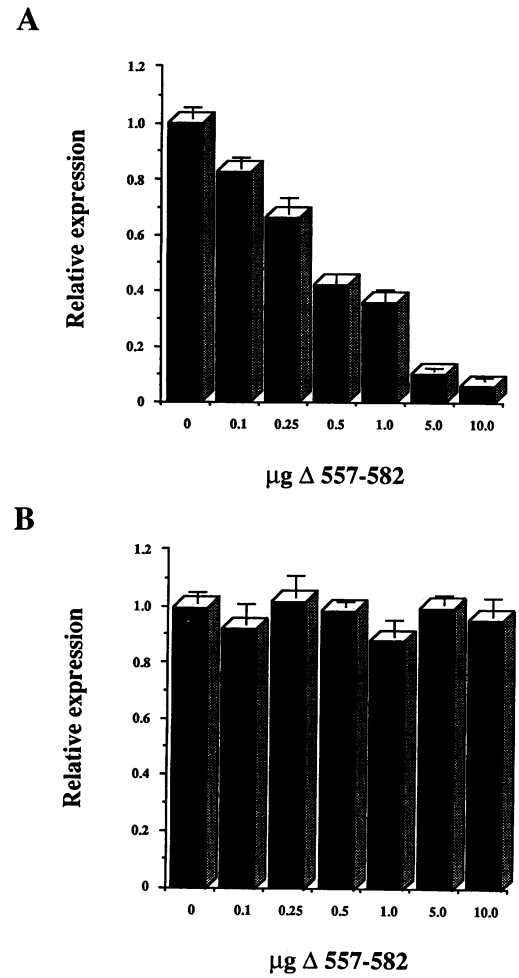


FIG. 6. Effect of Δ557–582 on IE1 transactivation of DE gene expression. We cotransfected 10^6 Sf9 cells with 1 μg of p39CATQ− (A) or p39CAT (B) plus 0.1 μg pNheIE1 and the indicated amounts of pΔ557–582. At 24 h posttransfection, cell lysates were prepared and analyzed for CAT activity. The averages of the results of three independent experiments are plotted as the amount of repression relative to the amount of pΔ557–582 cotransfected. Error bars are indicated above the columns.

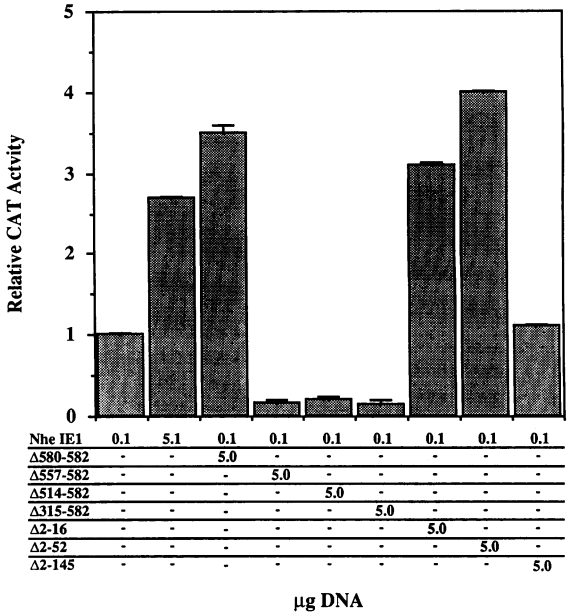


FIG. 7. *trans*-dominant inhibition of DE gene expression by IE1 mutants. We cotransfected 10^6 Sf9 cells with 1 μg of p39CATQ−, 0.1 μg of pNheIE1, and the indicated amounts of IE1 mutants. At 24 h posttransfection, cell lysates were prepared and analyzed for CAT activity. The average CAT activity of three separate experiments is illustrated, with standard deviations above columns.

Thus, we chose to investigate whether the *trans*-dominant mutant Δ557–582 also interferes with IE0-mediated transactivation. Sf9 cells were cotransfected with p39CATQ−, a constant amount of pGC123 (an IE0-expressing plasmid), and different amounts of pΔ557–582. CAT expression was assayed 1 day after transfection (Fig. 8). Unexpectedly, Δ557–582 did not interfere with IE0-mediated transactivation; conversely, increasing the amount of pΔ557–582 augmented expression of p39CATQ−. CAT expression was stimulated approximately two- to fourfold by increasing amounts of pΔ557–582. In a control experiment in which various amounts of pΔ557–582 were cotransfected with an IE0 reporter plasmid (IE0CAT), we found no significant effect on CAT expression (data not shown).

In summary, only mutant forms of IE1 that retained the N-terminal activation domain could *trans*-dominantly interfere with IE1-mediated transactivation. Furthermore, these mutants interfered only with the enhancer-dependent activation function of IE1; they had no effect on transactivation in the absence of the *hr*5 enhancer. Lastly, the *trans*-dominant mutant Δ557–582 augmented IE0-mediated transactivation.

DISCUSSION

Structural arrangement of the IE1 protein. Many viruses have genes that encode multifunctional *trans*-acting factors

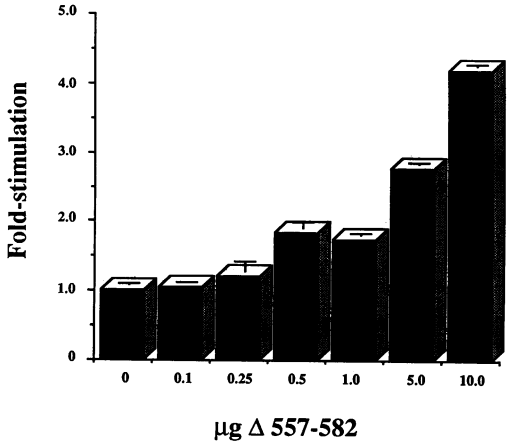


FIG. 8. Effect of Δ557–582 on IE0-mediated enhancer-dependent transactivation. We cotransfected 10^6 Sf9 cells with 1 μg of p39CATQ−, 0.5 μg pGC123, and the indicated amounts of pΔ557–582. At 24 h posttransfection, cell lysates were prepared and analyzed for CAT activity. The averages of the results of three independent experiments are plotted as the amount of stimulation relative to the amount of pΔ557–582 cotransfected. Error bars are indicated above the columns.

TABLE 1. Summary of IE1 and deletion mutant activities

Protein	Amino acid deleted[a]	Transactivation of 39K and IE1	Inhibition of IE0CAT	DNA binding	*trans*-dominant interference
IE1	0	+	+	+	NA[b]
Δ580–582	C2	+	+	+	NA
Δ557–582	C25	−	−	−	+
Δ514–582	C68	−	−	−	+
Δ315–582	C267	−	−	−	+
Δ2–16	N15	+	+	+	NA
Δ2–52	N51	+	+	+	NA
Δ2–145	N144	−	+	+	−

[a] C, carboxy; N, amino.
[b] NA, not applicable.

that both positively and negatively modulate gene expression. As evidenced by the functional dissections of several of these factors, many contain multiple genetically separable and functionally autonomous domains (13, 33, 43). We have analyzed deletion mutants of the Ac*M*NPV IE1 regulatory protein by using transient-expression and DNA-binding assays. A summary of our results is shown in Table 1. These data suggest that IE1 has at least two distinguishable domains, an N-terminal region essential for transactivation of IE1 and 39K and a C-terminal region(s) required for DNA-binding activity and inhibition of IE0.

Sequence comparisons between the Ac*M*NPV and Op*M* NPV IE1 (AcIE1 and OpIE1) ORFs have shown that they are approximately 63% homologous (41). Interestingly, the N-terminal one-third of the predicted ORFs shares the smallest amount of sequence similarity (22% for amino acids 1 to 132 in OpIE1 and 1 to 150 in AcIE1). However, even though the primary sequences have diverged, these two regions have maintained a highly acidic profile. The N termini of OpIE1 and AcIE1 have net charges of −13 and −14, respectively. The C-terminal two-thirds of the IE1 ORFs share 55% overall homology, with the greatest similarity (70%) between amino acids 459 to 538 in OpIE1 and 481 to 558 in AcIE1. On the basis of these structural similarities, it has been hypothesized that the N-terminal region of IE1 may contain an acidic activation domain and that the more highly conserved C terminus may contain a specificity domain.

Acidic activation region. Acidic activation domains have been identified in a number of transactivators, including yeast GCN4 (21) and GAL4 (2, 24) and herpes simplex virus VP16 (43). The results presented here suggest that a 93-amino-acid region between residues 52 and 145 may be required for transactivation. This region contains 16 negatively charged amino acids and has a net negative charge of −12. The region between residues 1 and 52 contains an additional 6 acidic residues which may be a component of the activation domain. It has been shown that sequential deletions of negatively charged amino acids within an acidic activation domain result in diminution of activation function (11). Consistent with that observation, Δ2–16 transactivated gene expression 1.5- to 2-fold greater than Δ2–52 did.

IE1 is the only known baculovirus protein that transactivates both minimal promoters and promoters linked to *hr* enhancers. We have analyzed the effects of IE1 deletions on transactivation of the IE1 and DE 39K (with and without an enhancer) promoters by transient-cotransfection assays in uninfected cells. Our results show that the putative acidic activation domain is required for stimulation of all three constructs. We propose that this region of IE1 functions as a general activation domain that may interact with a cellular transcription factor(s). However, we do not rule out the possibility that within this region, IE1 contains separate subdomains that are required for enhancer-independent and -dependent transactivation.

DNA-binding region. If our working hypothesis is correct, the N-terminal activation domain should require a second region that confers specificity for target genes. The transient-cotransfection assays we conducted showed consistently that deleting 25 or more amino acids from the C terminus resulted in loss of transactivation function. This could have been the result of either the loss of a second activation domain or, more consistent with our results, the deletion of a specificity domain. This domain may confer specificity to target genes directly (in the form of a DNA-binding domain) or indirectly (by serving as an interface for protein-protein interactions with factors bound to target genes). Although the EMSA used in this study does not distinguish between these two possibilities, it did allow us to investigate whether IE1 was a component of the protein-DNA complex.

Prior to this study, it had been demonstrated that cells transfected with IE1 DNA contained an *hr5* enhancer-specific DNA-binding activity (14). We believed that if the DNA-binding domain was contained within the C-terminal portion of the IE1 polypeptide, deletions from the N terminus that do not affect the binding domain should result in sequentially smaller IE1 gene products that form faster-migrating complexes in an EMSA. Our results demonstrate that IE1 forms a complex with *hr5*; however, we do not rule out the possibility of additional cellular proteins bound within these complexes.

Although sequence similarity was not found with any other DNA-binding domain, the secondary structure of the C-terminal amino acids from positions 485 to 582, as predicted from Chou and Fasman analysis (8), suggests that it may fold into a helix-loop-helix (9, 44)-like DNA-binding motif. Helix-loop-helix motifs consist of a basic stretch of amino acids that precede two alpha helices that are separated by a loop of variable length. Protein-protein interactions mediated by the alpha helices of two helix-loop-helix-containing proteins result in the formation of dimers. It is believed that dimerization results in the juxtapositioning of the basic domains of two monomers to yield a dimeric molecule capable of binding DNA. Since there is dyad symmetry in dimeric DNA-binding proteins, it is common for them to bind to palindromic sequences, such as those found in the *hr5* enhancer (15). Recent evidence from our laboratories has suggested that the IE1 gene product binds in the form of a dimer directly to the *hr5* enhancer (27; unpublished observations). The predicted secondary structure of this region and its demonstrated requirement for binding lead us to believe that the C terminus of IE1 contains a DNA-binding domain.

Inhibitory domain of IE1. It has been demonstrated by transient-expression assays that IE1 down-regulates the expression of two other baculovirus IE genes (4, 29). We chose to study the effect of IE1 deletions on the expression of an IE1/IE0 reporter plasmid. Our results suggest that the transactivating and inhibitory activities of IE1 are located in different regions of the polypeptide. The key observation supporting this hypothesis was shown by the mutant Δ2–145, which could not transactivate gene expression but down-regulated IE0 expression as efficiently as wild type IE1 did. These results also suggest that inhibition of IE0 expression

may require a functional DNA-binding domain, since deletions within the binding domain ablated inhibitory activity.

These data are consistent with a previously suggested model (29), which predicted that IE1 may play a role in E gene modulation during viral infection. Since IE1 has been shown to regulate a number of different gene classes, we propose that baculovirus gene regulation requires multiple associations between different domains of IE1 and other viral and/or cellular factors.

***trans*-dominant interference by IE1 mutants.** As noted above, expression of a nonfunctional mutant form of a regulatory protein may interfere with the function of the wild-type molecule (13, 20, 33, 43). *trans*-dominant mutants provide information about interactions between different *trans*-acting factors and can be useful tools in the characterization of regulatory pathways. We found that nonfunctional C-terminal mutants act as *trans*-dominant repressors of wild-type IE1. Interestingly, they only abrogate the ability of IE1 to transactivate an enhancer-linked gene. These data suggest that although IE1 requires the same domain(s) for both enhancer-dependent and -independent activation, there may be a fundamental difference between these two pathways.

Our data also show that only nontransactivating IE1 mutants that retain the putative acidic activation domain are able to interfere with wild-type IE1. We had initially predicted that since Δ2–145 was able to bind *hr5*, it may interfere directly with wild-type IE1 by competing for a binding site(s). Unexpectedly, it exhibited no interference activity. We speculate that the acidic activation region is involved in protein-protein interactions with an enhancer-dependent cellular factor. Our results are consistent with the "squelching" model of transcriptional interference (38). According to this theory, very high concentrations of a transactivator or a mutant form lacking a specificity domain may sequester unbound target proteins away from cognate genes and subsequently squelch or reduce transcription of those genes. In our system, we believe that IE1 mutants that are not targeted to the *hr5* enhancer retain the ability to interact with transcription factors away from the enhancer and, in doing so, squelch p39CATQ− expression.

We had previously shown that IE0 transactivates p39CATQ− (with enhancer) but does not transactivate p39CAT (without enhancer) (29). We speculated that if IE1 and IE0 transactivate p39CATQ− via the same pathway, Δ557–582 should interfere with both transactivators. Contrary to our expectation, Δ557–582 augmented transactivation of the reporter plasmid. We do not believe that Δ557–582 augments IE0-mediated transactivation by enhancing IE0 levels, since it had no effect on IE0CAT expression from the pGCAT reporter (Fig. 4, compare lanes 2 and 9). Although a number of regulatory mechanisms are possible, we hypothesize (i) that augmentation may occur via an interaction between Δ557–582 and IE0, which results in a more active form of IE0, or (ii) that Δ557–582 interacts with a cellular factor that normally competes with IE0 for a binding site on *hr5*, and hence Δ557–582 functionally sequesters this factor away from the target gene.

The analyses discussed thus far do not directly demonstrate that the various IE1 mutants are correctly localized within the transfected cell. The observed regulatory and DNA-binding properties of IE1 lead us to assume that it is a nuclear protein. IE1 has a predicted molecular mass of 70 kDa. This large size suggests that it may have a nuclear localization signal. Deletion of a signal sequence may result in improperly localized mutant polypeptides. If we assume that *trans*-dominant interference is mediated by direct competition for a common factor(s), proper localization would most probably be essential. The interference assays (Fig. 7) show that all three nontransactivating C-terminal polypeptides expressed interference activity, suggesting that they are probably targeted to the same subcellular compartment(s) as wild-type IE1. Lastly, although the N-terminal mutant Δ2–145 did not express interference activity, it did repress IE0CAT expression in vivo (Fig. 4, lane 7), suggesting that this mutant is also localized properly.

Use of transfection assays to probe IE1 function. The transient-expression experiments described here provide a reasonable approach to study IE1 gene function. Although the assay has aided us in studying many of the regulatory properties of IE1, the data are limited by the fact that they were obtained in the absence of viral infection. Various attempts to delete the IE1 gene from the Ac*M*NPV genome have failed, suggesting that it may be an essential gene (unpublished results). As a result, extension of this type of mutational analysis to the context of the viral genome has not been possible. Work in our laboratories is currently focusing on the development of inducible promoters for the construction of conditional-lethal mutant viruses, which may enable us to test the various IE1 mutants in the complex environment of the infected cell.

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