JOURNAL OF VIROLOGY, Nov. 1991, p. 5820–5827
0022-538X/91/115820-08$02.00/0

Vol. 65, No. 11

Baculovirus gp64 Gene Expression: Analysis of Sequences Modulating Early Transcription and Transactivation by IE1

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Received 20 May 1991/Accepted 24 July 1991

Expression of the baculovirus major envelope glycoprotein gene (gp64) is regulated by transcription from both early and late promoters. To characterize the early promoter and identify sequences involved in the regulation of gp64 early transcription, promoter-reporter gene fusions were generated from the *Orygia pseudotsugata* nuclear polyhedrosis virus gp64 promoter and were analyzed by transient expression in uninfected insect cells. For these analyses, 5′ deletion mutations were constructed in the gp64 upstream regulatory region. Larger promoter constructs were functional in uninfected *Lymantria dispar* cells, indicating that transcription from the gp64 early promoter required no additional viral gene products. Deletion analysis of the gp64 upstream region revealed several regulatory regions. These included a putative negative regulatory element between −319 and −166 nucleotides (nt) and multiple positive regulatory elements between −166 and −77 nt. Deletion of the TATA box located between −77 and −62 nt resulted in the loss of transcriptional activity. Cotransfections of reporter constructs and a plasmid containing a baculovirus transcriptional transactivator gene (*Autographa californica* nuclear polyhedrosis virus IE1) resulted in transcriptional transactivation of all constructs containing an intact TATA box. These data demonstrate that sequences upstream of the gp64 TATA box are not essential for IE1 transactivation and that only 34 nt upstream of the early transcription start site were necessary for basal levels of transcription and for transactivation by IE1. Function of the gp64 early promoter was also examined in cell lines from *Spodoptera frugiperda* and *Drosophila melanogaster*.

The *Baculoviridae* is a family of insect pathogenic viruses with large (88 to 150 kbp), double-stranded supercoiled DNA genomes. They are characterized by a complex infection cycle which produces two structurally and functionally distinct virion phenotypes (for a review, see reference 3). Virions of the polyhedron-derived virus (PDV) phenotype acquire an envelope in the nucleus and are subsequently occluded in large polyhedron-shaped occlusion bodies (termed polyhedra). In contrast, virions of the budded virus (BV) phenotype are not occluded and acquire an envelope by budding through the virus-modified plasma membrane at the cell surface. The BV phenotype serves to spread the infection from cell to cell within an infected individual. The major envelope glycoprotein (gp64) of the BV phenotype is encoded by the virus and is required for endocytosis of the virus into host insect cells (31). The genes encoding the gp64 proteins from two baculoviruses, *Orygia pseudotsugata* nuclear polyhedrosis virus (OpMNPV) and *Autographa californica* nuclear polyhedrosis virus (AcMNPV), have been located and sequenced (2, 33), and the temporal nature of gp64 expression has been examined. Although most baculovirus structural proteins are expressed as late genes, gp64 gene expression is regulated by both early and late promoters. Early transcripts initiate just downstream of a TATA box within a sequence (CAGT) which is conserved at the transcription start sites of several baculovirus early genes (2). After the onset of DNA replication, several gp64 late transcripts initiate farther upstream, within and around two baculovirus late promoter core motifs (ATAAG). Although numerous recent studies have dealt with the identification and characterization of the basic unit of the baculovirus late promoter (6, 22–24, 30, 32), baculovirus early promoters have only recently received attention (8, 11, 21, 29).

Baculovirus early promoters have been classified as immediate early and delayed early on the basis of their presumed requirements for other viral gene products for transcription (15). Immediate-early genes were believed to be transcribed immediately upon entry of the virus, and delayed-early genes were believed to be transcribed only after some prior viral gene expression. Experimentally, immediate-early genes were classified as genes capable of transcriptional activity in transient expression assays in uninfected insect cells (15, 21). Delayed-early genes were believed to be inactive in uninfected cells in the absence of other required viral gene products. However, the immediate-early and delayed-early distinctions are currently unclear because transcriptional activity was recently detected from the prototype delayed-early promoter (AcMNPV, 39 kDa) in uninfected insect cells (29). We previously demonstrated that gp64 early transcripts and the gp64 protein are present in infected cells prior to DNA replication (2, 5). In the current studies, we examined transcription from the gp64 early promoter in uninfected insect cells to identify upstream regions important for transcription early in infection. To specifically address these questions, we initiated studies to (i) determine whether the gp64 early promoter is transcribed in uninfected cells, (ii) identify upstream sequences which are required for or modulate levels of early transcription, and (iii) determine whether transcription of the gp64 early promoter is stimulated by a baculovirus transcriptional transactivator protein, IE1. We also examined the expression of the OpMNPV gp64 early promoter in three insect cell lines: *Lymantria dispar* (Ld), *Spodoptera frugiperda* (Sf9), and *Drosophila melanogaster* (Dm1).

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For these studies, we generated gp64 promoter-reporter gene fusions in which the gp64 upstream region and a portion of the gp64 open reading frame (ORF) were fused to a bacterial chloramphenicol acetyltransferase (CAT) gene (p64CAT plasmids). To identify regulatory regions in the gp64 upstream sequence, a series of 5′ deletion mutations was generated and constructs were analyzed by transient expression in several cell lines. To confirm that CAT activity resulted from translation of mRNAs which initiated at the authentic gp64 early start sites, cytoplasmic RNAs were isolated from transfected cells and analyzed by primer extension analysis. In addition, transactivation by the baculovirus IE1 gene product was examined by cotransfecting several cell lines with p64CAT constructs and a plasmid containing a baculovirus IE1 gene.

MATERIALS AND METHODS

Construction of promoter-CAT fusions. To construct gp64 promoter-reporter fusions, we used a plasmid containing the entire gp64 gene and approximately 1,560 nucleotides (nt) of upstream untranslated sequence in a cloned 3.3-kbp *Hin*dIII-*Bam*HI fragment from OpMNPV (Fig. 1a and b, p64HdB) (2). The p64HdB plasmid was digested with *Bam*HI, filled in with Klenow polymerase, and religated to remove the *Bam*HI site. The majority of the gp64 ORF (nt 21 to 1527) and a small portion of the 3′ untranslated region were removed and replaced with a *Bam*HI linker in the following manner. The modified p64HdB plasmid was digested with *Nco*I, followed by digestion with exonuclease III and then *Acc*I, and filled in with Klenow polymerase. A *Bam*HI linker was then added, and the resulting plasmids were subcloned. A subclone which contained the *Bam*HI linker inserted at nt 21 of the gp64 ORF was identified by DNA sequencing and designated p64HdB21 (Fig. 1c). The p64HdB21 plasmid was then digested with *Bam*HI, and a 770-bp *Bam*HI fragment containing the CAT ORF (18) was cloned into the *Bam*HI site at position +21. The resulting plasmids contained the CAT ORF in either the same orientation as the gp64 ORF (Fig. 1d, p64CAT-1560) or in the opposite orientation (p64CAT-1560−). Thus, plasmid p64CAT-1560 contains approximately 1,560 nt of upstream untranslated sequence, 21 nt of the gp64 ORF fused to the CAT ORF, and 195 nt of the 3′ untranslated region of gp64 (which includes three polyadenylation signals). A similar construct which contains only 319 nt of upstream sequence was constructed by digesting the p64CAT-1560 plasmid partially with *Hin*cII and completely with *Sma*I and subcloning the excised fragment into the *Hin*cII site of a Bluescribe plasmid (pBS−). The resulting construct was designated p64CAT-319 (Fig. 1e). An analogous construct was subcloned from the p64CAT-1560− plasmid (which contains the CAT ORF in the opposite orientation), and the resulting plasmid was designated p64CAT-319−.

5′ deletions of the gp64 upstream region. For functional analysis of upstream regions, a series of 5′ deletion subclones was generated from plasmid p64CAT-319 (Fig. 1e). The gp64 upstream region was unidirectionally deleted from the 5′ upstream end (*Hin*cII site) by digesting the plasmid with *Kpn*I and *Xba*I (sites in the pBS− multiple cloning site) and then digesting it with exonuclease III (17). The ends of the deleted plasmids were made flush with S1 nuclease and Klenow polymerase, a 12-nt *Xho*I linker was added, and the plasmids were ligated and subcloned. The precise location of the deletion and *Xho*I linker in each selected deletion subclone was determined by DNA sequencing. The number of each deletion construct indicates the number of nucleotides upstream of the gp64 translation start, with the A of the gp64 ATG designated as +1 (Fig. 1e and f and Fig. 2). (Note: The ATG was used as a reference point because the gp64 gene contains at least five mRNA start sites that vary in position by more than 100 nt.) Thus, deletion subclone p64CAT-158 contains 158 nt of sequence upstream of the gp64 ORF, whereas deletion subclone p64CAT-77 contains only 77 nt of upstream sequence.

Transfections, RNA isolations, primer extension analyses, and CAT assays. Ld-652Y, Sf9, and Dm1 cells were propagated as described previously (2, 25, 28). For isolation of cytoplasmic RNAs, 3.5×10^6 cells were seeded on 60-mm-diameter petri plates and transfected with 20 μg of plasmid DNA by calcium phosphate precipitation (13) as modified for insect cells (28). For larger constructs containing approximately 1,560 nt of upstream sequence, the amount of plasmid DNA was increased proportionately so that equimolar amounts of plasmid DNAs were added to each well. Cells were incubated at 27°C for 4 h in the transfection buffer-DNA mixture which was subsequently replaced with fresh medium and incubated at 27°C for 48 h. Total cytoplasmic RNA was isolated from transfected cells by the hot phenol method (26). For primer extension assays, 45 μg of each cytoplasmic RNA was annealed to a 5′-end-labeled oligonucleotide complementary to the 5′ end of the CAT ORF (Fig. 2). The oligonucleotide was 5′ end labeled, and primer extension analyses were performed as described previously (1, 2). Sizes of primer extension products were determined by comparison to a DNA sequencing ladder of a modified pBS vector plasmid (4).

For analysis of transient expression by CAT assay, 1.2×10^6 cells were seeded on 35-mm-diameter wells of a multiwell tissue culture plate and transfected as described above, but with 10 μg of each plasmid DNA. In addition to promoter-reporter constructs, cells were also transfected with two control plasmids: pBS (Stratagene) and pCAT, a plasmid containing the CAT ORF (18) but no gp64 sequences. For cotransfections, 10 μg of each DNA was added to 1.2×10^6 cells (each well therefore received 20 μg of DNA). Each p64CAT promoter construct was cotransfected with either control DNA (a pBS− vector plasmid) or a plasmid (pIE1) which contains the gene for the AcMNPV transcriptional transactivator IE1 (15, 16) under the control of its own promoter. Transfected cells were incubated at 27°C for 48 h and then were removed from the plates by being gently scraped with a rubber policeman. Cells were gently pelleted and resuspended in 50 μl of 100 mM Tris (pH 7.8) and then lysed by three freeze-thaw cycles. Cell debris were pelleted, and the supernatants containing the cell extracts were used for CAT assays. CAT assays were performed by the two-phase fluor diffusion assay (19). For each CAT assay, 20 μl of each cell extract (4.8×10^5 cell equivalents) was used in a 250-μl reaction mixture containing 100 mM Tris (pH 7.8), 1 mM chloramphenicol, and 0.1 μCi [^{14}C]acetyl coenzyme A (4 mCi/mmol; New England Nuclear). Reactions were performed in miniscintillation vials and each reaction mixture was overlaid with 5 ml of Econofluor (New England Nuclear). Acetylation of chloramphenicol was measured by direct scintillation counting at 2 h (Ld and Sf9 cell extracts) or 18 h (Dm1 cell extracts) after the start of the reaction. Reactions containing 0.01, 0.05, or 0.1 U of CAT (Sigma) were prepared and used as positive controls to confirm the linearity of the assay at the times sampled. For each experiment involving a single insect cell type (Fig. 3), the counts per minute of acetylated chloram-

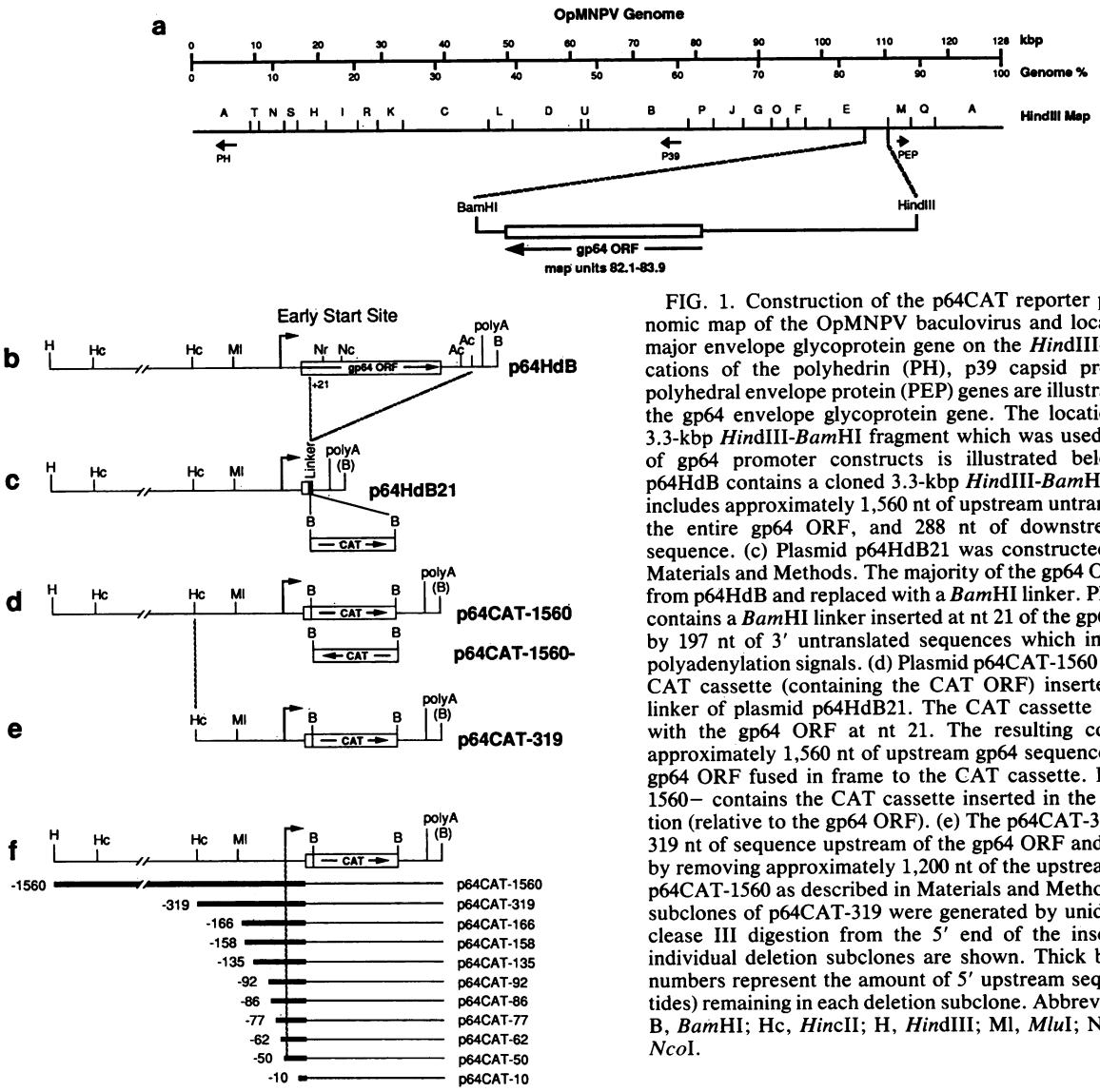


FIG. 1. Construction of the p64CAT reporter plasmids. (a) Genomic map of the OpMNPV baculovirus and location of the gp64 major envelope glycoprotein gene on the *Hin*dIII-E fragment. Locations of the polyhedrin (PH), p39 capsid protein (P39), and polyhedral envelope protein (PEP) genes are illustrated in relation to the gp64 envelope glycoprotein gene. The location of the cloned 3.3-kbp *Hin*dIII-*Bam*HI fragment which was used for construction of gp64 promoter constructs is illustrated below. (b) Plasmid p64HdB contains a cloned 3.3-kbp *Hin*dIII-*Bam*HI fragment which includes approximately 1,560 nt of upstream untranslated sequence, the entire gp64 ORF, and 288 nt of downstream untranslated sequence. (c) Plasmid p64HdB21 was constructed as described in Materials and Methods. The majority of the gp64 ORF was removed from p64HdB and replaced with a *Bam*HI linker. Plasmid p64HdB21 contains a *Bam*HI linker inserted at nt 21 of the gp64 ORF, followed by 197 nt of 3′ untranslated sequences which include three gp64 polyadenylation signals. (d) Plasmid p64CAT-1560 contains a 770-bp CAT cassette (containing the CAT ORF) inserted in the *Bam*HI linker of plasmid p64HdB21. The CAT cassette is fused in frame with the gp64 ORF at nt 21. The resulting construct contains approximately 1,560 nt of upstream gp64 sequence and 21 nt of the gp64 ORF fused in frame to the CAT cassette. Plasmid p64CAT-1560− contains the CAT cassette inserted in the opposite orientation (relative to the gp64 ORF). (e) The p64CAT-319 insert contains 319 nt of sequence upstream of the gp64 ORF and was constructed by removing approximately 1,200 nt of the upstream sequence from p64CAT-1560 as described in Materials and Methods. (f) 5′ deletion subclones of p64CAT-319 were generated by unidirectional exonuclease III digestion from the 5′ end of the insert. Some of the individual deletion subclones are shown. Thick bars and negative numbers represent the amount of 5′ upstream sequence (in nucleotides) remaining in each deletion subclone. Abbreviations: Ac, *Acc*I; B, *Bam*HI; Hc, *Hin*cII; H, *Hin*dIII; Ml, *Mlu*I; Nr, *Nar*I; and Nc, *Nco*I.

phenicol obtained from extracts of cells transfected with different constructs are directly compared. To determine the degree of variability between transfections, duplicate transfections were performed for each construct and the range of variability is indicated in Fig. 3.

RESULTS AND DISCUSSION

To determine whether the gp64 early promoter is expressed in uninfected insect cells and to identify regions important in regulation of the gp64 early promoter, complete p64CAT and p64CAT deletion subclones were transfected into uninfected insect cells and assayed for transcriptional (CAT) activity. For these studies, three insect cell lines were used: Ld, the lepidopteran cell line in which the OpMNPV virus replicates; Sf9, another lepidopteran cell line; and Dm1, a dipteran cell line. p64CAT constructs were expressed in uninfected cells of all three cell lines, indicating that in uninfected insect cells, transcription from the gp64 early promoter requires no other viral gene products and is not limited to the host in which the OpMNPV virus normally replicates. In addition, examination of the deletion mutants indicated that although the levels of activity varied among the different cell lines, the overall trends observed within each cell line were similar (Fig. 3, solid bars). Constructs containing the greatest amount of upstream sequence (1,560 and 319 nt) did not produce the highest levels of CAT activity but appeared to be transcribed at lower levels than constructs containing only 166 and 158 nt of upstream sequence (Fig. 3, −1560 and −319 versus −166 and −158, solid bars). The dramatic increase in CAT activity observed after deletion of sequences between −319 and −166 nt suggests the presence of a negative regulatory element in this region. The highest levels of CAT activity were observed with constructs which contained 166 and 158 nt of upstream sequence. An almost stepwise decrease in transcriptional activity was observed as sequences between −166 and −77 were deleted. No transcriptional activity was detected from constructs containing 62, 50, and 10 nt of upstream sequence as was the case for control constructs with the CAT ORF inserted in the opposite orientation (p64CAT-1560− and p64CAT-319−) and for control plasmids pBS and pCAT

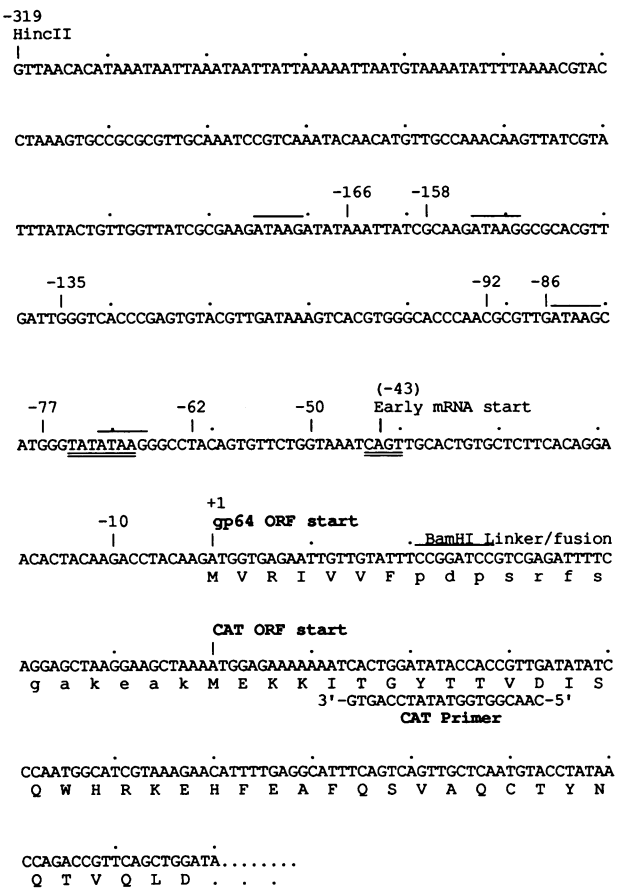


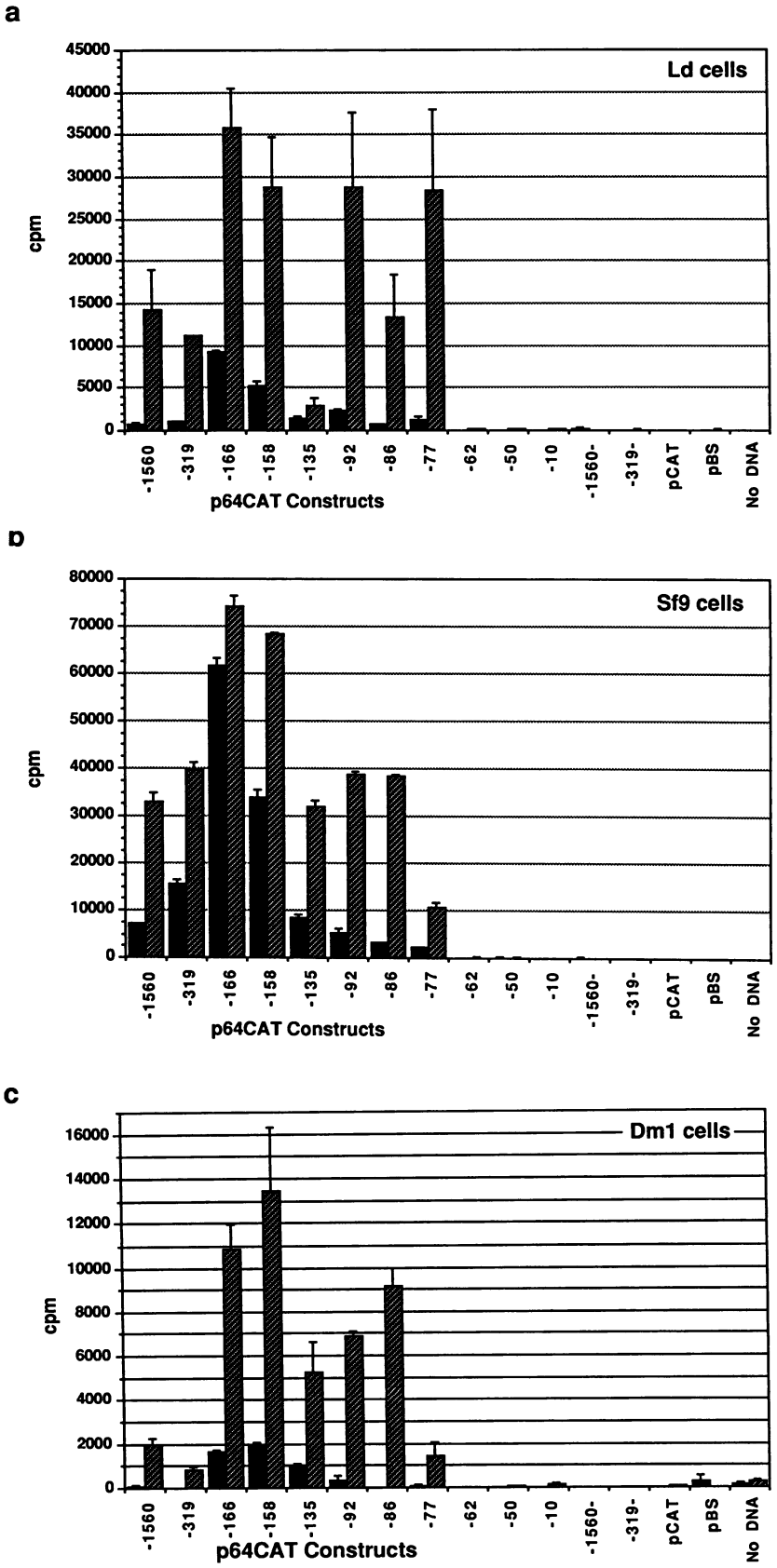
FIG. 2. Nucleotide sequence of the p64CAT-319 upstream region and the location of deletion subclones. Sequences upstream of the gp64 ORF and the location of the gp64 and CAT ORF fusion are illustrated. Locations of deletion subclones are indicated above the sequence (negative numbers, relative to the gp64 ORF). Late baculovirus promoter core sequences (ATAAG) are overlined, and conserved sequences associated with early transcription are double underlined. The locations of the translation start codons for the gp64 ORF and the CAT ORF are indicated, and amino acid sequences are shown below the nucleotide sequence. Amino acids shown in lowercase result from translation of the *Bam*HI linker and the upstream region of the CAT cassette in this fusion. The location and sequence of the CAT primer used for primer extension analysis are shown below the CAT ORF.

(Fig. 3). Thus, the general trend observed in all cell lines was a reduction in CAT activity as sequences between −166 and −77 nt were deleted from the 5′ end. The largest reduction in CAT activity (four- to eightfold in lepidopteran cells) was observed when sequences between −166 and −135 nt were deleted. Subsequent deletions between −135 and −77 nt further reduced transcription but less dramatically (Fig. 2 and 3). Transcription was abolished when the sequence containing the TATA box (−77 to −62 nt) was deleted (Fig. 2 and 3; −77 versus −62).

Because the regulation of some baculovirus early genes (9, 15, 21, 29) is influenced by a baculovirus transcriptional transactivator, we examined the effect of the AcMNPV transcriptional transactivator IE1 on transcription from the gp64 early promoter in uninfected cells. For these studies, cells were cotransfected with each p64CAT construct and plasmid pIE1, a plasmid containing the AcMNPV IE1 gene (15). Cotransfection with pIE1 stimulated p64CAT transcription in all cell lines and from all promoter constructs which were otherwise transcribed in the absence of IE1 (Fig. 3, solid versus hatched bars). No significant transcriptional activity was detected when pIE1 was cotransfected with constructs p64CAT-62, p64CAT-50, p64CAT-10, or control plasmids. A potential result, incorporated into the design of this experiment, was the identification of upstream sequences which mediated transactivation by IE1. The deletion of a region containing a sequence element which mediated IE1 transactivation would inactivate the potential for IE1 stimulation. Cotransfection with pIE1 would then result in a level of transcriptional activity equal to the level observed when cotransfecting with the control pBS plasmid. However, IE1 stimulation was observed with all deletion constructs which contained a TATA box (Fig. 2 and 3; constructs p64CAT-77 and larger), suggesting that the effect of IE1 is either (i) directly or indirectly associated with sequences within the upstream 77 nt or (ii) indirect and not mediated by sequence elements. It is possible that such transactivation could involve the interaction of IE1 with a protein(s) which binds to the TATA box (e.g., TFIID) or the entire RNA polymerase II transcription complex. The lack of sequence specificity (upstream of the TATA box) for IE1 transactivation appears similar to observations on the adenovirus E1A protein which does not bind to specific upstream sequences but may functionally distinguish between different TATA box sequences (12, 20, 27, 34). We are currently examining this phenomenon in greater detail.

IE1 transactivation of the gp64 promoter in Ld, Sf9, and Dm1 cells also suggests that the AcMNPV IE1 promoter (on the pIE1 plasmid) is active in these three cell types. The relative stimulation of constructs by IE1 was not equivalent for different promoter deletion subclones. For example, in Sf9 cells, constructs −166, −158, −135, and −92 were stimulated approximately 1.2-, 2-, 4-, and 8-fold, respectively, by IE1 (Fig. 3b). Also, for individual deletion clones, stimulation by IE1 was not the same when compared in different cell lines (Fig. 3; compare −166 stimulation by IE1 in Ld, Sf9, and Dm1 cells). One possible explanation for these data is that different cell lines may have different levels of cellular transcription factors which interact with IE1, thus resulting in different degrees of stimulation. However, IE1 consistently stimulated transcriptional activity from all deletion constructs containing an intact TATA box. Levels of CAT activity detected from reporter constructs varied in the different cell lines. The highest levels of CAT activity were observed in Sf9 cells, followed by Ld cells. Overall, levels of transcriptional activity were low in Dm1 cells. When Dm1 cells were used for cotransfections of the control plasmid and construct p64CAT-86 or p64CAT-77, no CAT activity was detected even though the same constructs were transcriptionally active in Ld and Sf9 cells. Transcription from these constructs was, however, transactivated in Dm1 cells when these constructs were cotransfected with pIE1. The data from Ld and Sf9 cells (Fig. 3a and b) suggest that constructs p64CAT-86 and p64CAT-77 may be transcriptionally active in Dm1 cells, but the levels of activity were below our limits of detection. However, it is clear that transcription from these constructs was transactivated by IE1. Recently, the IE1 gene was identified from the OpMNPV genome (29). Preliminary experiments using the OpMNPV IE1 gene for cotransfections (not shown) resulted in transactivation similar to that observed with the AcMNPV IE1 gene.

In addition to demonstrating transcriptional activity, it is essential to demonstrate that transcripts from plasmid con-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pCAT
pBS
No DNA
p64CAT Constructs

FIG. 3. Relative CAT activity detected from p64CAT constructs transfected into Ld (a) Sf9 (b), and Dm1 (c) cells. Individual p64CAT promoter constructs are indicated on the X axis, with deletion subclones indicated by negative numbers (see Fig. 1f and 2). Negative controls include transfections with the pCAT and pBS plasmids as well as mock transfections (No DNA). Solid bars represent average CAT activity from p64CAT plasmids cotransfected with control plasmid (pBS). Hatched bars represent average CAT activity from p64CAT plasmids cotransfected with the pIE1 plasmid. Relative CAT activity is indicated on the Y axis as counts per minute (above background) of ^{14}C-acetylated chloramphenicol. Error bars represent the range of activities detected from duplicate transfections.

structs initiate at the transcription start sites utilized in OpMNPV-infected cells. Therefore, we transfected Ld and Sf9 cells with selected p64CAT constructs, isolated cytoplasmic RNAs, and used primer extension analysis to examine the accuracy of transcription initiation. When uninfected Sf9 and Ld cells were transfected with gp64 promoter constructs containing 319, 166, and 77 nt of gp64 upstream sequences, primer extension products of 135, 136, and 137 nt were detected (Fig. 4). This corresponds to transcription initiation at the A, G, and T of the conserved CAGT. Since early transcription initiation was originally reported from a single start site (the A of the CAGT) in OpMNPV-infected Ld cells, it is likely that this heterogeneity represents stuttering of the reverse transcriptase at the end of the mRNA rather than a heterogeneity of mRNA start sites. More importantly though, transcription initiated within the conserved CAGT sequence in all constructs which were transcriptionally active in CAT assays (-319, -166, and -77). Transfection with the promoter construct containing only 62 nt of upstream sequences resulted in no detectable primer extension product, as was the case when a control Bluescribe plasmid (pBS−) was transfected. Thus, transcription initiation from the gp64 early start site in uninfected Sf9 and Ld cells confirms that gp64 is expressed as an early gene. In minimal promoter constructs created by 5′ deletions, transcription initiated accurately when only 77 nt of the upstream sequences remained, but transcription was abolished when a 15-nt sequence (containing the TATA box) was deleted. Therefore, these data show that the 77 nt upstream of the gp64 ORF contain sufficient information to specify accurate transcription initiation.

In addition, the TATA box appears to be an important component of this minimal early promoter. Primer extension analysis of RNAs from cells cotransfected with pIE1 and p64CAT plasmids indicates that the gp64 transcripts are initiating accurately in different cell types (Sf9 and Ld) and when stimulated by IE1 (Fig. 4). While many baculovirus early genes have TATA boxes within the 5′ upstream regions, others do not. In addition, the CAGT motif, which is conserved at the transcription start sites of gp64, IE1, IEN, and the 39,000-molecular-weight promoter (1, 7, 10, 14, 16, 29), is not found at the start sites of all baculovirus early genes. Thus, regulatory sequences associated with baculovirus early genes may be quite variable, perhaps reflecting

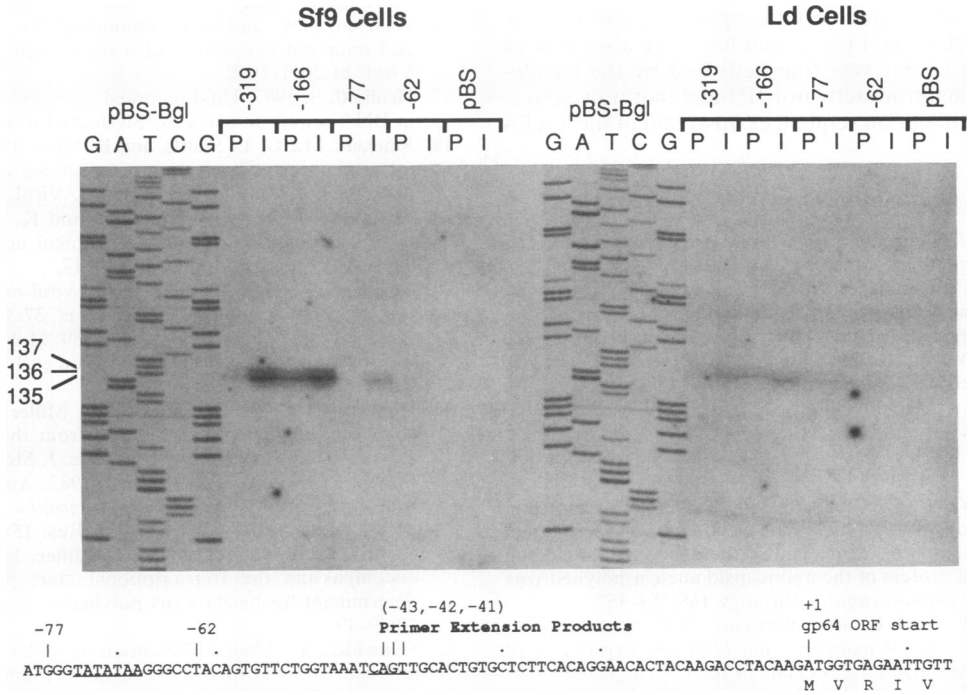

FIG. 4. Primer extension analysis of transcripts from transfected Sf9 and Ld cells. Selected p64CAT promoter constructs (Fig. 1f) were used to transfect uninfected Sf9 and Ld cells. Cytoplasmic RNAs were isolated from transfected cells and analyzed by primer extension analysis by using a 20-nt CAT primer (Fig. 2). Numbers above the lanes indicate the promoter construct used for transfections. Numbers represent the amount of gp64 upstream sequence remaining, and pBS represents the control pBS− plasmid (Stratagene) containing no insert. Each promoter construct was cotransfected with either the control pBS− plasmid (P) or a plasmid containing the baculovirus IE1 gene pIE1 (I). Sizes of primer extension products were determined by comparison to a sequencing ladder (pBS-Bgl). Locations of the conserved TATA box and CAGT motif (underlined) are indicated along with mRNA start sites (Primer Extension Products) on the sequence below.

the utilization of many different aspects of the insect cell's transcriptional regulatory apparatus. Similar to the results reported here, several recent studies (8, 11, 29) of baculovirus early genes show transcriptional activity from constructs containing only the TATA box and sequences downstream. In addition, point mutations in the conserved CAGT (at the transcription start site of IEN) substantially reduced CAT activity in transient assays (8), although initiation accuracy was not examined. These data suggest that in baculovirus early promoters containing both a TATA box and a start site CAGT motif both elements are important. We are currently using synthetic minimal promoter constructs and a linker scanning analysis to elucidate the relative roles of these elements.

In summary, we have identified regions containing upstream sequence elements which regulate transcription from the gp64 early promoter in several insect cell lines. Two putative regulatory regions which modulate the levels of gp64 early transcripts were identified. A region between nt −319 and −166 appears to contain a negative regulatory element and the region between −166 and −77 appears to contain multiple positive regulatory elements. In addition, 5′ deletions flanking the TATA box identify the 5′ boundary of a minimal promoter (containing only 34 nt upstream of the transcription start site) capable of basal levels of accurate transcription initiation. This suggests that the TATA box is an important element of the gp64 early promoter. For many vertebrate genes, the TATA box is considered to be the minimal unit of the RNA polymerase II promoter. However, because gp64 and a number of other baculovirus early genes have a conserved motif (CAGT) at or near their transcription start sites (2), this CAGT motif may also participate in the regulation of early gp64 expression. In addition to demonstrating function and accuracy of the gp64 early promoter in uninfected cells of several insect cell lines, we also showed that this early promoter was transactivated by the baculovirus transcriptional transactivator IE1 and that transactivation was not dependent on sequences upstream of the TATA box.

ACKNOWLEDGMENTS

We thank Linda Guarino for providing the pIE1 plasmid and Reginald McParland for synthetic oligonucleotide synthesis. We also thank David Theilmann for the gift of a cloned OpMNPV IE1 gene while the manuscript was in preparation.

G.W.B. was supported in part by National Research Service Award AI07886 from the National Institutes of Health. This work was also supported by grants AI21973 and AI31130 from the National Institutes of Health and by project 1250 of the Boyce Thompson Institute.

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