A Baculovirus *gp64* Early Promoter Is Activated by Host Transcription Factor Binding to CACGTG and GATA Elements

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The early promoter of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus *gp64* gene is active when transfected into several insect cell lines and does not require viral gene products for transcription in uninfected cells. Because previous studies have shown that the *gp64* early promoter is activated above basal levels in uninfected cells, host transcription factors are likely to play a role in *gp64* activation at early times postinfection. By using nuclear extracts from uninfected Sf9 cells for electrophoretic mobility shift analysis of *gp64* regulatory regions, host nuclear proteins were shown to bind specifically to the upstream regulatory region of the *gp64* early promoter. Host factor binding was mapped to a 24-bp sequence centered approximately 35 bp upstream of the TATA box. Two consensus eukaryotic transcription factor-binding site motifs, GATA and CACGTG, were identified within the 24-bp sequence. Competition assays using oligonucleotides containing either a GATA or a CACGTG motif and similar oligonucleotides with point mutations in these sites showed that each site is required for binding host transcription factors. To investigate the functional significance of host factor binding to GATA and CACGTG motifs, constructs containing point mutations in these motifs were examined in transient expression assays. Mutations in either or both GATA and CACGTG sites decreased reporter activity in transient expression assays, suggesting that binding of host transcription factors to these motifs is important in transcriptional regulation of the *gp64* early promoter.

The *Baculoviridae* is a family of large double-stranded DNA viruses that are pathogenic for certain groups of insects. The baculovirus genome is a covalently closed circular DNA ranging in size from approximately 80 to 180 kbp. After virus entry and uncoating, the viral genome is transcribed and replicated in the nucleus. Gene expression is regulated in a cascade fashion (19, 25, 43, 44) with early gene expression preceding DNA replication and late gene expression. Early transcription is mediated by host RNA polymerase II (20, 22, 27, 28), and functional studies suggest that many early promoters contain a functional TATA box (1, 4, 16, 24, 37, 50) as do most RNA polymerase II promoters of higher eukaryotes. The TATA box motif of eukaryotic promoters is recognized by the TATA-binding protein, a component of transcription factor IID, which provides a nucleation site for assembly of the RNA polymerase II complex. Like TATA box motifs from insects and other higher eukaryotes, those from baculovirus early promoters direct the site of transcript initiation. While the TATA box supports transcription at basal levels (1, 4, 16, 24, 50), other elements are required for activation. In addition to the TATA box, many baculovirus early promoters contain a conserved sequence (CAGT) at or near the transcription start site (3, 9, 13, 25, 26, 34, 50, 51), and a similar consensus sequence has been described for insect genes (7, 12, 29). Although the role of the conserved start site sequence is not fully resolved, the start site CAGT sequence is important for efficient basal transcription initiation but does not appear to be essential for transcription in the presence of a TATA box (1).

Transcription from most baculovirus early genes appears to be activated by the interaction of both host and viral transcrip-

tion factors with viral regulatory sequence elements. The best characterized of the viral transcription factors is the transactivator, IE-1, which is known to stimulate transcription of a number of early genes (4, 25, 26, 31, 32, 37, 50). IE-1 stimulates transcription from basal promoter elements and also activates transcription through viral enhancers. Additional viral transcription factors that have been identified in transient expression assays include IE-0, IE-N (also known as IE-2), PE-38, and LEF-2 (8–10, 13, 32, 40, 51). Many studies of viral early promoters have examined the role of viral gene products in transcriptional activation, but little work has focused on the interactions of host transcription factors with baculovirus promoter elements. Recently, Krappa and coworkers (33) provided the first demonstration of a lepidopteran nuclear protein binding to a viral sequence. In that study, a *Spodoptera frugiperda* nuclear protein, designated SfNP-1, was shown to bind to a consensus GATA site in the upstream regulatory region of the *pe-38* gene.

The baculovirus *gp64* gene encodes a 64- to 67-kDa envelope glycoprotein which is a major structural component of the envelope of the budded form of the virus (3, 45, 53, 56). GP64 is a pH-dependent membrane fusion protein and is required for viral entry into the host cell by endocytosis (5, 36, 54, 55). Expression of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus (OpMNPV) *gp64* gene is regulated by both early and late promoters (3). Early transcription initiates from a single site located 43 nucleotides upstream of the *gp64* coding region, and late transcripts initiate from consensus late promoter motifs (TAAG) located farther upstream. Transcription from the *gp64* early promoter can be detected at least 6 h prior to DNA replication in OpMNPV-infected *Lymantria dispar* cells (3, 6). In addition, the OpMNPV *gp64* early promoter is active in *L. dispar*, *S. frugiperda* (Sf9), and *Drosophila melanogaster* (Dm1) cell lines in the absence of viral proteins. Functional analysis of early promoter mutations (1, 4) suggests

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that the *gp64* early promoter can be divided into a basal promoter region and an upstream activating region. In transient expression assays, 5' deletions were used to identify upstream sequences that activate *gp64* transcription above basal levels in uninfected insect cells. Such studies of *gp64* and other early promoters suggest that early in infection, baculovirus early genes are recognized and activated by host transcription factors.

In the present study, we examined binding of nuclear proteins to the upstream regulatory region of the OpMNPV *gp64* gene. Using electrophoretic mobility shift analysis (EMSA) in combination with site-directed mutagenesis, and functional assays for transcription, we demonstrated binding of cellular transcription factors to a 24-bp upstream region containing two consensus binding site sequences: GATA and CACGTG. GATA motifs were first recognized as erythroid-specific *cis*-regulatory elements in vertebrate hematopoietic cells (17, 39) and have recently been described as transcription factor-binding sites in lepidopteran insect RNA polymerase II genes (49) and in the baculovirus *pe-38* gene (33). The CACGTG motif is a core recognition sequence for a large group of transcription factors known as the basic region/helix-loop-helix/leucine zipper (b-HLH-zip) proteins (21). In the current study, we show that nuclear proteins from *S. frugiperda* bind specifically to GATA and CACGTG motifs in the *gp64* early promoter. Point mutations in either the GATA or the CACGTG motif prevent binding of host transcription factors and reduce transcriptional activity from the early promoter. Mutations in both sites further reduce transcriptional activation. These results suggest that during initial stages of infection, binding of insect transcription factors stimulates transcription from the baculovirus *gp64* early promoter.

MATERIALS AND METHODS

Plasmids, probes, and competitors for EMSA. For initial EMSA experiments, probe and competitor DNAs containing sequences from the *gp64* early promoter were prepared from the p64CAT-166 construct. This construct contains 123 bp of sequence upstream of the early transcription start site, the 43-bp 5' untranslated leader, and 21 bp from the 5' end of the *gp64* open reading frame fused in frame, via a *Bam*HI linker, to a chloramphenicol acetyltransferase (CAT) reporter gene (4). To generate a labeled DNA probe for initial EMSA experiments, the p64CAT-166 plasmid was digested with *Xho*I and *Bam*HI, and the 198-bp promoter-containing fragment (Fig. 1B) was isolated from an agarose gel and purified with Mermaid Glassfug (Bio 101, Inc.). The 198-bp *Xho*I-*Bam*HI fragment was end labeled by filling in with Klenow polymerase and [³²P]dGTP as described previously (2). To reduce nonspecific binding, smaller probes were generated by isolating and end labeling fragments from p64CAT-120 constructs. These constructs are similar to p64CAT-166 but have 46 bp removed from the 5' end. The probe derived from p64CAT-120 is designated -120wt (5'-agctcccgtcgaACGTTGATAAAGTCACGTGGGCACCCAACGCG-3'), and similar probes containing binding site mutations are designated -120ΔGATA (5'-gactcccgctcgaACGTtctcgAAAGTCACGTGGGCACCCAACGCG-3') and -120ΔCACGTG (5'-agctcccgtcgaACGTTGATAAAGTCACGgGCGGCACCCAACGCG-3'). (Uppercase letters indicate baculovirus sequence, and lowercase letters indicate nonbaculovirus sequence. The underlined sequence indicates locations of binding site mutations.) Construction of the plasmids containing these sequences is described below.

Unlabeled competitor DNAs were prepared in the following

manner. To generate 5' deletions in the upstream promoter region, the p64CAT-166 plasmid was digested with either *Bst*EII or *Mlu*I, filled in with Klenow polymerase, ligated to *Xho*I linkers, cut with *Xho*I, gel purified, and religated. Viral sequences in the resulting deletion constructs are illustrated in Fig. 1B. Plasmid DNAs were linearized with *Pvu*II and used as competitors. Plasmids containing TATA mutations were generated by the following protocol. A plasmid, p64CAT-166ΔTAGA, containing the p64CAT-166 construct in which an *Xba*I site was inserted at the TATA box (5'-GtcTA~~g~~ATAAGGG-3') was digested with *Xba*I, then overdigested with S1 nuclease, filled in with Klenow polymerase, and reannealed. The resulting construct contains a discrete 12-bp deletion of the TATA box (5'-TTGATAAGCATGGGTATATAAGGGCC-3') was changed to 5'-TTGATAAGC-----GGGCC-3') and is designated p64CAT-166ΔTATA-12 (Fig. 1B, -166Δ12). 5' deletion subclones from the p64CAT-166ΔTATA-12 promoter were generated and prepared for competition experiments as described above for the p64CAT-166 plasmid. As additional controls for EMSA experiments, we used a previously described plasmid containing a 43-nucleotide basal promoter construct (wt43; see reference 1) and a pBS plasmid DNA (Stratagene).

For EMSA competition with isolated DNA fragments, plasmid p64CAT-166 or p64CAT-135 (Fig. 1B, -166 or -135, respectively) was digested, and DNA restriction fragments were purified. Sequences 5' and 3' of the *Rsa*I (-120), *Pml*I (-104), and *Ban*I (-98) restriction sites were examined in EMSA experiments (Fig. 3A). The 5' and 3' *Rsa*I sequences were isolated by digesting plasmid p64CAT-135 with *Rsa*I. Fragments with sizes of 748 and 273 bp were isolated from agarose gels and purified by using Spin-X filtration units (Costar). The 748-bp fragment contains sequences 5' of the *Rsa*I site (Fig. 3A, 5' *Rsa*) while the 273-bp fragment contains sequences 3' of the *Rsa*I site (Fig. 3A, 3' *Rsa*). The 5' and 3' *Pml*I sequences were similarly isolated by digesting p64CAT-166 with *Pml*I and *Pvu*II and then purifying 201-bp (Fig. 3A, 5' *Pml*) and 278-bp (Fig. 3A, 3' *Pml*) fragments from agarose gels. Sequences 5' and 3' of the *Ban*I site were isolated by digesting p64CAT-135 with *Ban*I and purifying 509-bp (Fig. 3A, 5' *Ban*I) and 840-bp (Fig. 3A, 3' *Ban*I) fragments from agarose gels.

For more-specific competition in EMSA, oligonucleotides containing promoter sequences were synthesized. Complementary oligonucleotides, 5'-agcTTGATAAAGTCACGTGGCACCCA-3' and 5'-agcTTGGGTGCCCCACGTGACTTTATCA-3' were annealed in a buffer containing 100 mM NaCl, 10 mM Tris (pH 7.9), 1 mM EDTA at a concentration of 8 pmol of each oligonucleotide per μl for 5 min at 90°C and then slowly cooled to 37°C overnight. (Baculovirus DNA sequences are shown in uppercase letters; lowercase letters at the 5' ends indicate the addition of a *Hind*III site.) This double-stranded oligonucleotide corresponds to an upstream sequence from -116 to -94, contains both the GATA and CACGTG sites, and is designated wt23mer. Smaller complementary oligonucleotides containing only the GATA site (5'-TACGTTGATAAAGTCA-3' and 5'-TGACTTTATCAACGTA-3') or the CACGTG site (5'-AAAGTCACGTGGGCAC-3' and 5'-ATGCCACGTGACTTT-3') were also prepared. These 16-mers were annealed as described above and are designated GATA-16 and CACGTG-16, respectively. Complementary oligonucleotides containing mutations in each of these sites were also made. A 16-mer containing four point mutations within the GATA motif (5'-TACGTtctcgAAAGTCA-3') is designated ΔGATA-16, and a 23-mer with point mutations in the CACGTG binding site sequence (5'-TGATA

FIG. 1. Regulatory region of *gp64* early promoter construct (p64CAT-166) used for detection of host nuclear DNA-binding proteins. (A) Sequences upstream of the OpMNPV *gp64* open reading frame (ORF) in plasmid construct p64CAT-166 are illustrated, with the locations of restriction sites, promoter elements, and consensus transcription factor-binding site motifs (GATA and CACGTG) indicated. The translation start site (boxed ATG) is designated as position +1. The TATA box (−74) and transcription start site CAGT sequence (−43) are also boxed. Uppercase sequences represent baculovirus DNA, and lowercase sequences represent linker DNA. GATA and CACGTG transcription factor-binding sites described in the text are boxed above the sequence. (B) Probe and competitor fragments used for analysis of binding by Sf9 DNA-binding proteins are illustrated below a diagram of the *gp64* upstream regulatory region in plasmid p64CAT-166. For initial EMSA, the *XhoI*-*BamHI* fragment (Probe) was end labeled and incubated with nuclear extract from uninfected Sf9 cells. Baculovirus sequences used as unlabeled competitor DNAs are illustrated below the probe fragment (−166, −135, and −92). Similar competitor DNAs containing a 12-bp deletion of the TATA box (Δ12) are illustrated at the bottom. The location of the 12-bp deletion is indicated on the diagram (Δ-12). Results of competition with each fragment are indicated to the right of each competitor and are shown in Fig. 2.

AAGTCATGatGGaACCCA-3') is designated ΔCACGTG-23. A commercial double-stranded oligonucleotide (TFIID oligo; Promega) containing a binding site for the TATA-binding protein was used as a negative control for oligonucleotide competition experiments.

Nuclear extracts and EMSA. A continuous *S. frugiperda* cell line, Sf9, was maintained at 27°C in TNMFH medium as described previously (1). For preparation of nuclear extracts, cells were grown to high densities (2×10^6 to 4×10^6 cells per ml) and harvested in log-phase growth. Nuclear extracts (4.4 to 9 mg of protein per ml) were prepared as described previously (1, 27). Nuclear extract (7 mg of protein per ml) was prepared from an *L. dispar* cell line (IPLB-LD-652Y) under the same conditions.

For each binding reaction, 1 to 20 fmol of end-labeled DNA

and 5 μ l of 4 $\times$ buffer (80 mM HEPES (N-2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid [pH 7.9], 80 mM KCl, 24 mM MgCl₂, 4 mM dithiothreitol, 50% glycerol) plus 1 to 2 μ g of poly(dI-dC) (Pharmacia) were combined in a total volume of 17 μ l. To this solution, 3 μ l of Sf9 nuclear extract, which was freshly diluted with water just prior to use, was added so that the final concentration was 5 μ g of protein per 20 μ l of reaction mixture. When *L. dispar* nuclear extract was used, the final concentration of protein was 10 μ g per 20 μ l of reaction mixture. Binding reaction mixtures were incubated for 20 to 30 min at either 0 or 27°C. When included, competitor DNAs were added at 50- to 900-fold molar excess. After incubation, DNA-protein complexes were immediately loaded into either 5 or 10% high-ionic-strength acrylamide gels, which had been

prerun at 100 V for 1.5 to 3 h. Samples were electrophoresed in Tris-glycine buffer for 4 to 5 h at 30 or 35 mA at 4°C as described previously (14). Following electrophoresis, gels were dried under vacuum or between cellophane (Research Products International) and analyzed by autoradiography.

Transient expression analysis. To investigate the effects of binding on function of the *gp64* early promoter, we generated 5' deletions and point mutations in the binding sites of the *gp64* early promoter and examined transcriptional activity. To examine function by deletion mutagenesis, we used a series of previously generated 5' deletion constructs (4), and additional nested deletions were generated by digestion with *XhoI* and either *RsaI* or *PmlI*. Ends were filled in with Klenow polymerase and religated. To discretely destroy the GATA- and CACGTG-binding sites, we used a modification of long primer-unique site elimination mutagenesis (41) to insert multiple point mutations. The GATA site in p64CAT-120 was changed from GTTGATAA to GTctcgAA; this construct is designated -120ΔGATA. Two point mutations were inserted into either the 5' or 3' side of the CACGTG site of p64CAT-120, destroying the CACGTG motif with fewer mutations than in the ΔCACGTG-23 oligonucleotide. Two nucleotides at either the 5' or 3' end of the CACGTG motif were changed to atCGTG or CACGac. These constructs are designated -120atCGTG and -120CACGac, respectively. Finally, a construct combining point mutations in both the GATA site (same as that described above) and in the 3' nucleotides of the CACGTG was generated. This construct is designated -120ΔGATA/CACGac. All cloned mutations were confirmed by DNA sequencing. Promoter-reporter plasmids containing point mutations in GATA and/or CACGTG were examined in transient expression assays. Transfections and CAT assays were performed as described previously, with minor modifications (1, 4). For transfections, each plasmid (10 μg) was used to transfect 2.2×10^6 Sf9 cells. Cells were incubated for 48 h, harvested in 1 ml of TNMFH medium, gently pelleted, resuspended in 100 μl of 1 × phosphate-buffered saline (PBS), lysed by three cycles of freeze-thawing, and heat inactivated at 65°C for 60 min. After cellular debris was pelleted, the extracts were frozen at -80°C until use in CAT assays. For the 5' deletion series, 5% of each extract was added to a cocktail containing 1 μCi of [³H]acetyl coenzyme A and 5 mM chloramphenicol. Control plasmids containing no promoter (pCAT) or no CAT cassette (pBS) were included as negative controls. For the -120 mutation series, 20% of each extract was used for CAT assays. Transfections and CAT assays were performed in triplicate.

RESULTS

EMSA. In previous studies of the *gp64* early promoter, functional analyses of promoter mutations have shown that the early promoter can be conceptually divided into a basal promoter and upstream activation regions. A basal promoter consisting of sequences from the TATA box through the conserved start site CAGT sequence was shown to specify accurate transcription initiation at minimal or basal levels (1). Deletions in the upstream region showed that transcription from the *gp64* basal early promoter is influenced by upstream sequence elements (4). To examine interactions between these upstream promoter elements and host transcription factors, we selected a *gp64* promoter-reporter construct (p64CAT-166) that was previously shown to exhibit relatively high levels of transcriptional activity (4). In initial EMSA experiments, a 198-bp *XhoI*-*Bam*HI fragment from this construct (Fig. 1) was end labeled and used as a probe for binding proteins from Sf9

FIG. 2. Analysis of binding of Sf9 nuclear proteins to the *gp64* regulatory region by EMSA. The probe and competitor DNAs used for EMSA are illustrated in Fig. 1B. Free probe, which was not incubated with Sf9 nuclear extract, is indicated in lane 1, and the probe incubated with nuclear extract but no competitor is shown in lane 2. Specific competition by competitor DNAs is shown in lanes 3 to 8. Competition experiments with fragments containing 5' deletions (-166, -135, and -92) are shown in lanes 3 to 5, and similar experiments with fragments containing TATA box deletions (Δ12; see Fig. 1B) are shown in lanes 6 to 8. The location of the specific protein-DNA complex is indicated by an arrowhead. Control competitor DNAs consisting of a basal promoter (wt43) and a plasmid vector (pUC) did not compete for the specific complex (lanes 9 and 10, arrowhead).

nuclear extracts. To locate upstream DNA sequences that specifically bound host proteins, unlabeled DNAs containing 5' deletions were used as competitors in binding reactions for EMSA. Competitor DNAs are shown in Fig. 1B as -166, -135, and -92, with negative numbers indicating the number of base pairs remaining upstream of the *gp64* translation start site. To determine whether the protein binding was independent of TATA box interactions, similar DNAs but with the TATA box deleted were used as competitors. In these constructs, the TATA box was discretely removed by a 12-bp deletion (Fig. 1B). A pUC plasmid and a plasmid containing the 43-bp *gp64* basal promoter (wt43; see reference 1) were also used as controls in competition experiments.

When the 198-bp *XhoI*-*Bam*HI probe was used for protein binding, numerous protein-DNA complexes were observed (Fig. 2, lane 2). However, when a plasmid containing the -166 *gp64* promoter sequence was used as an unlabeled competitor for protein binding and EMSA, specific competition of a single DNA-protein complex was observed (Fig. 2, lane 3, arrowhead). Constructs containing 166 bp (-166) and 135 bp (-135) of upstream sequence competed for binding of this complex (Fig. 2, lanes 3 and 4). However, the construct containing only 92 bp of upstream sequence showed no specific competition for this complex (Fig. 2, lane 5, arrowhead). The same result was observed when similar constructs containing deletions of the TATA box were used as competitors (Fig. 2, lanes 6 to 8). Neither the 43-bp basal promoter (wt43) nor the pUC plasmid competed for this specific complex (Fig. 2, lanes

FIG. 3. Mapping of the binding site region by EMSA. (A) An *XhoI*-*Bam*HI fragment (Probe) was end labeled and incubated with Sf9 nuclear extract and used for EMSA. Unlabeled competitors are shown below the diagram of the *gp64* regulatory region. The sequence of the 36-bp *BstEII*-*MluI* fragment is indicated below the diagram. To map binding within the 36-bp *BstEII*-*MluI* fragment, promoter plasmids were digested with restriction enzymes that cleave within this 36-bp region, and 5' or 3' fragments were isolated and used in EMSA (see Materials and Methods). Sequences used as competitors are indicated as 5' and 3' restriction fragments. (B) Results of EMSA using competitors from the -92 to -166 region are shown in lanes 2 to 4. Controls (no competitor) and a pBS plasmid competitor are shown in lanes 1 and 5, respectively. (C) Results of EMSA using competitors containing sequences 5' and 3' of restriction sites *RsaI* (lanes 1 and 2), *BanI* (lanes 3 and 4), and *PmlI* (lanes 7 and 8) are shown. Controls show complete competition by p64CAT-166 (-166; lane 6) and no competition in the presence of no competitor or a pBS plasmid (No Competitor, pBS; lanes 5 and 9, respectively).

9 and 10). In addition to deletion constructs, specific regions of *gp64* promoter DNA were subcloned and used in EMSA competition experiments. The region from -166 to -92 contains restriction enzyme sites at positions -166, -135, and -92 (Fig. 3A). Therefore, to confirm the above results,

plasmids containing baculovirus DNA from this region were used as competitors with the same labeled probe. Plasmids containing either the *XhoI*-*MluI* fragment (-166 to -92) or the *BstEII*-*MluI* fragment (-135 to -92) competed for factor binding. However, a plasmid containing the *XhoI*-*BstEII* fragment (-166 to -135) did not compete (Fig. 3B). Thus, with deletion constructs and defined upstream regions as competitors, the binding site was mapped to a 36-bp region between the *BstEII* (-135) and *MluI* (-92) sites.

To more specifically map the binding site, we used three restriction enzyme sites which are found within this 36-bp sequence: *RsaI* (-120), *PmlI* (-104), and *BanI* (-98) (Fig. 3A). Plasmid DNAs containing the promoter region were digested with each restriction enzyme (*RsaI*, *PmlI*, or *BanI*), and fragments 5' or 3' to the restriction enzyme sites within the promoter region were isolated and used as competitors in EMSA. The competitor fragments are identified as either 5' or 3' of the restriction sites (Fig. 3A). With the same 198-bp *XhoI*-*Bam*HI probe DNA as in the previous experiment, Fig. 3C shows that DNA sequences 5' of the *RsaI* site did not compete for binding, whereas sequences 3' of *RsaI* resulted in unambiguous competition. Sequences 5' of the *BanI* site gave partial competition, but sequences 3' of the *BanI* site did not compete. When DNA was digested with *PmlI*, neither 5' nor 3' sequences competed for binding (Fig. 3C), suggesting that this restriction site is within or very near the identified binding site. These results indicate that a binding site lies between the *RsaI* and *MluI* sites and that sequences within or near the *PmlI* and *BanI* sites may be critical for binding.

Oligonucleotide competitors. To more precisely map the binding sites for the lepidopteran transcription factors, double-stranded oligonucleotide competitors were used in EMSA. To reduce background in EMSA gels, we used smaller DNA probes containing only 32 bp of baculovirus sequence (*RsaI*-*MluI*, Fig. 4A) and observed a decrease in the number of nonspecific complex bands and clearer resolution of specific bands (Fig. 4B and 5B). A number of complexes were consistently observed when no competitor was added (Fig. 4B and 5B, lanes 1). An oligonucleotide competitor containing 23 bp of the baculovirus DNA sequence centered around the *PmlI* site (-116 to -94) (Fig. 4A and 5A, wt23mer) competed for six major DNA-protein complexes (Fig. 4B and 5B, lanes 2). The specificity of binding is described below. A negative control oligonucleotide (TFIID24mer) of similar size but containing a binding site for the transcription factor IID complex did not compete, even at 100-fold molar excess (data not shown).

Because attempts to map the binding site at high resolution by DNase footprinting have thus far been unsuccessful, we used an alternative strategy for further mapping. The 23-bp sequence was examined for similarities to known transcription factor binding sites. Two binding site similarities were identified: a potential GATA site [(A/T)GATA(G/A)] (17) and a potential CACGTG-binding site motif (21). To examine these sites for participation in complex formation, we synthesized oligonucleotides containing each of these sites alone and oligonucleotides containing multiple point mutations in each of these two potential binding site sequences (Fig. 4A and 5A). The selected mutations were based on mutations that were previously shown to interfere with transcription factor binding (17, 35, 42, 49). The oligonucleotides shown in Fig. 4A and 5A were used as competitors in EMSA to examine the GATA and CACGTG sequences for specific complex formation (Fig. 4B and 5B, respectively).

Whereas the wt23mer oligonucleotide competed for six major DNA-protein complexes (Fig. 4B, lane 2), a smaller

FIG. 4. EMSA of a GATA motif using synthetic oligonucleotide competitors with wild-type and mutant GATA sites. (A) Sequences of probes and competitor DNAs used for EMSA are indicated below a map of the *gp64* regulatory region. One strand of each double-stranded probe and double-stranded synthetic oligonucleotide competitor is shown. Probe fragments were end labeled and incubated with an Sf9 nuclear extract. For labeled probes, fragments were isolated from three -120 constructs: -120wt, -120ΔGATA, and -120ΔCACGTG. Uppercase sequence indicates baculovirus DNA, and lowercase indicates nonbaculovirus DNA. Substitution mutations are boxed. The 23-bp wild-type competitor oligonucleotide (wt23mer) contains both the GATA and CACGTG motifs. The 16-bp wild-type competitor oligonucleotide (GATA-16) contains the GATA, but not the CACGTG, motif. To confirm GATA-specific binding, a similar 16-bp competitor containing four point mutations in the GATA site (ΔGATA-16, boxed nucleotides) was used. (B) EMSA of protein binding at the GATA motif. EMSA experiments using the three different probes (indicated above each panel) are shown left to right. Oligonucleotide competitors are indicated directly above the lanes; these competitor DNAs were used at 100-fold molar excess. On the left, the locations of GATA-specific complexes are indicated by arrowheads. The wt23mer also competed for two additional complexes that were not GATA specific (filled circles).

16-bp oligonucleotide that contained only the GATA site, but not the CACGTG site, competed for only four of those complexes (Fig. 4B, GATA-16, lane 3, arrowheads). In contrast, a similar 16-bp oligonucleotide that contained four point mutations in the GATA site (Fig. 4A, ΔGATA-16) did not compete for these four complexes (Fig. 4B, lane 4), indicating that discrete destruction of the GATA site eliminated binding. This result also was confirmed by the use of two additional DNA probes. When a probe lacking the GATA site was used for EMSA (Fig. 4A, probe -120ΔGATA) the same four complexes did not form (Fig. 4B, compare lanes 1 and 5, arrowheads). In contrast, the four complexes did form when a probe that lacked the CACGTG site was used (Fig. 4B, -120ΔCACGTG, compare lanes 1 and 9, arrowheads). Thus, four of the six specific complexes that formed in this region required the GATA sequence.

Because the 16-mer containing the GATA site did not compete for two of the major specific complexes (Fig. 4B, lanes 2 and 3), we examined the possibility that these two complexes resulted from protein interactions with the CACGTG motif. Although the wt23mer competed for the two complexes (Fig. 5B, lane 2, arrowheads), a similar double-stranded oligonucleotide containing four point mutations at the CACGTG site (Fig. 5A, ΔCACGTG-23) did not compete for these complexes (Fig. 5B, compare lanes 2 and 3, arrowheads). In addition, a smaller 16-bp oligonucleotide containing the CACGTG site, but not the GATA site (Fig. 5A, CACGTG-16), competed for only these two complexes (Fig. 5B, compare lanes 1 and 4, arrowheads). When a DNA probe containing point mutations in the CACGTG motif was used (Fig. 5A, -120ΔCACGTG), the two specific complexes did not form (Fig. 5B, compare lanes 1 and 9, arrowheads), confirming that mutation of the CACGTG core motif disrupts binding. Similar results were obtained when a nuclear extract from *L. dispar* cells was used to examine protein binding at GATA and CACGTG sequences (data not shown).

Functional analysis of mutations in the GATA- and CACGTG-binding sites. Because binding alone does not indicate functional importance, we examined the effects of specific mutations in the GATA and CACGTG motifs on transient expression of *gp64* early promoter-CAT reporter gene constructs. For these experiments, the p64CAT-166 plasmid (4) was used as the reference reporter plasmid (Fig. 1). The binding sites were removed from *gp64* early promoter constructs by two methods: (i) a series of 5' deletions was constructed in the upstream sequence, and (ii) site-directed mutagenesis was used to insert point mutations into the GATA- and CACGTG-binding sites. The effects of 5' deletions through the promoter region are shown in Fig. 6A. On the basis of EMSA competition experiments, binding of nuclear factors requires the GATA and CACGTG core sequences, which are located between -120 and -104. A deletion mutation that removed sequences between -120 and -104 (removing all of the GATA site and half of the CACGTG site) reduced CAT activity to near basal levels (Fig. 6A, -120 versus -104). In addition, mutation of either the GATA site or the CACGTG site in the otherwise intact p64CAT-120 promoter resulted in an approximately 50% reduction in total transcriptional activity compared with that of the -120 wild-type construct (Fig. 6B). Removal of both sites reduced activity further, suggesting an additive effect from the double mutation. Thus, data from physical and functional analyses demonstrated that removal of the GATA and/or CACGTG sites results in a loss of host transcription factor binding and a corresponding reduction in transcriptional activity.

DISCUSSION

FIG. 5. EMSA of a CACGTG motif using synthetic oligonucleotide competitors with wild-type and mutant CACGTG sites. (A) Sequences of probes and competitor DNAs used for EMSA are indicated below a map of the *gp64* regulatory region. Probes are the same as those described in the legend to Fig. 4A. One strand of each double-stranded probe and double-stranded synthetic oligonucleotide competitor is shown. The 23-bp wild-type competitor oligonucleotide (wt23mer) contains both the GATA and CACGTG motifs. The 16-bp wild-type competitor oligonucleotide (CACGTG-16) contains the CACGTG, but not the GATA, motif. To confirm CACGTG-specific binding, a 23-bp competitor containing four point mutations in the CACGTG site (Δ CACGTG-23, boxed nucleotides) was used. (B) EMSA of protein binding at the CACGTG motif. EMSA experiments using the three different probes (indicated above each panel) are shown left to right. Oligonucleotide competitors are indicated directly above the lanes; these competitor DNAs were used at 100-fold molar excess. On the left, the locations of CACGTG-specific complexes are indicated by arrowheads, and GATA-specific complexes are indicated by filled circles.

Baculovirus genes expressed early after infection use host cell proteins for transcriptional activation. Although the host RNA polymerase II complex is known to be involved (20, 22, 27, 28), additional transcription factors that activate or modulate transcription are likely provided by both the incoming virus and the host. An expanding body of work now documents the effects of viral encoded transcription factors on early gene expression (1, 4, 8–10, 23, 25, 31, 32, 37, 50–52). In contrast to studies of viral transcription factors, host transcription factors that interact with viral early promoters have received very little attention. Krappa and coworkers (33) recently reported the first example of a protein from *S. frugiperda* cells that binds to a GATA motif upstream of a baculovirus early gene (*pe-38*) and designated this protein factor “*S. frugiperda* nuclear protein 1,” or SfNP-1. In the current study, we demonstrated specific protein binding to two motifs, a GATA motif and a CACGTG motif, in the OpMNPV *gp64* early promoter. In addition, we demonstrated by mutagenesis and functional assays that binding of host factors to these motifs is likely important for early transcriptional regulation.

Using oligonucleotide competitors and constructs containing discrete deletions in the TATA box, we demonstrated that binding of these proteins is independent of the TATA box sequence. In addition, previous studies (1, 4) have shown that this upstream region is not required for basal levels of transcription from the *gp64* promoter. These data indicate that the binding of host transcription factors to the GATA and CACGTG motifs is important for the activation of transcription, but these specific binding sites are not required for the TATA-dependent basal promoter activity, which was previously characterized (1, 4).

On the basis of the results of EMSA, we show that specific DNA-protein complexes map within a 24-bp region (–121 to –97) centered 35 bp upstream of the TATA box of the *gp64* early promoter. Protein binding was mapped to consensus GATA and CACGTG motifs. The critical nucleotides for binding at these sites have not yet been determined since we were unable to obtain footprinting data. However, analysis of the effects of point mutations within the GATA and CACGTG sequences by protein binding and transient expression assays indicates that mutation of a few nucleotides in either core motif disrupts binding and reduces transcriptional activation. Using small probes containing 32 bp of baculovirus sequence, we found that multiple complexes form between insect nuclear extracts and each of these sequence motifs, GATA and CACGTG. In addition, data from EMSA using probes containing mutations in either the GATA or CACGTG sites, indicate that the GATA and CACGTG complexes form independently.

By inserting mutations in the GATA- and CACGTG-binding sites of the *gp64* early promoter and examining the resulting transcriptional activity by transient expression analysis, we show that protein binding to these sites activates transcription from the *gp64* early promoter. Mutations which deleted or replaced nucleotides within these binding sites result in (i) loss of specific binding and (ii) substantial reductions ($\geq 50\%$) in transcriptional activation. While these results are consistent with results from a previous study (4), a more dramatic reduction in CAT activity was observed in the present study when sequences between –135 and –92 were deleted. These differences are likely due to the increased sensitivity of the CAT assays used in the current study. Elimination of binding at the GATA and CACGTG motifs did not reduce transcriptional activity to background levels since transcription

FIG. 6. Functional analysis of binding site mutations in the *gp64* early promoter. (A) Discrete 5' deletion mutations that remove the GATA and CACGTG motifs from a *gp64* early promoter-CAT construct are shown on the left. The location of the GATA and CACGTG motifs are indicated on the diagram by filled boxes. The GATA motif is centered at position -114, and the CACGTG motif is centered at position -104 from the translation start site. A plasmid containing a CAT cassette but no promoter sequences is shown as a negative control (pCAT). (B) The effect of point mutations in the GATA- and CACGTG-binding sites was examined by transient expression assays. On the left, constructs containing multiple point mutations in the GATA motif (-120 Δ GATA), the CACGTG motif (-120atCGTG and -120CACGac), and both (-120 Δ GATA/CACGac) are shown below the wild-type construct (-120). A construct containing only the TATA box and sequences downstream (-77) also is included for comparison. Locations of binding sites containing point mutations are indicated by open boxes. Filled boxes indicate wild-type binding sites. For both panels, the graphs on the right show relative CAT activity; bars represent mean cpm values (above background) from three transfections, and error bars represent standard deviations from the means.

from the basal promoter remains active. In addition, transcription from a construct containing point mutations in the GATA and CACGTG motifs was higher than that from a construct containing only the TATA box and sequences downstream (Fig. 6B, $-120\Delta\text{GATA/CACGac}$ versus -77), suggesting that additional sequences in the $-120\Delta\text{GATA/CACGac}$ construct also may play some role in activation of transcription from the *gp64* early promoter. Binding of additional host transcription factors in the upstream region also is suggested by the results of deletions immediately upstream of the GATA- and CACGTG-binding sites examined in this study. Deletion of sequences between nucleotides -166 and -120 results in significant reductions in transcriptional activity (Fig. 6A, constructs -166 through -120).

The consensus sequence (A/T)GATA(G/A) is recognized by a family of GATA proteins found in vertebrate blood cells, *D. melanogaster*, and fungi (39). These proteins are zinc-finger DNA-binding proteins. The nuclear magnetic resonance structure of the chicken GATA-1 protein complexed with DNA has recently been reported (38). A recent study (33) demonstrated binding of a host nuclear protein (designated, SfNP-1) to a GATA-binding site in the promoter region of the *Autographa californica* MNPV *pe-38* gene, although no effect on transcriptional activity was detected in cell culture. In addition, EMSA competition experiments suggested binding to GATA motifs in the *A. californica* MNPV *me53* and *35K* promoters (33). In the present study we detected sequence-specific protein binding to the GATA motif located at -114 in the OpMNPV *gp64* early promoter. In contrast to the previous study (33), we observed a decrease in transcription when the GATA motif was deleted or changed. Because deletions of regions from wild-type or mutant constructs containing additional GATA motifs (Fig. 1A, -153 and -85) resulted in decreased transcriptional activation (Fig. 6A and 6B), we cannot exclude the possibility that these other GATA sequences also may be involved in the regulation of *gp64* early transcription.

Mutation of the CACGTG sequence results in loss of host protein binding and in a reduction of transcriptional activation of the *gp64* early promoter. The CACGTG sequence has been identified as a regulatory sequence in at least two other groups of DNA viruses that are transcribed and replicated in the nucleus: adenoviruses and cytomegaloviruses (herpesviruses) (11, 30, 47). The CACGTG sequence serves as a core recognition sequence for a large group of transcription factors containing the following structure: basic region/helix-loop-helix/leucine zipper. Transcription factors in this group are found in many organisms from yeasts to plants and man and are known to regulate the genes of several viruses (21). These factors include the vertebrate major late transcription factor (MLTF; also known as the upstream stimulatory factor, USF), as well as Myc, Max, Myn, FIP, TFE3, TFEB, CP1, GFII, UEF, GBF, and CG-1 (21). Recognition of the core sequence (CACGTG) is specified by the basic region of the b-HLH-zip transcription factor, and single amino acid changes in the basic region are capable of altering the specificity of core binding (15). In addition to recognition of the core sequence, nucleotides immediately flanking the core are important in determining transcription factor-binding specificity (18). These transcription factors often bind as heterodimers, and the recognition site specificity may also vary on the basis of the subunits of the heterodimer. One of the most-studied transcription factors of this group is MLTF, which has been characterized as a heterodimer consisting of 43- and 44-kDa subunits (46, 48).

Because baculovirus early genes utilize the basic transcription apparatus of the host cell, it is likely that baculoviruses

also utilize the plethora of host cell nuclear transcription factors that are available. In the current study, we demonstrated binding of host transcription factors to GATA and CACGTG motifs within a 24-bp sequence. Binding of these factors stimulates transcription from the *gp64* early promoter. While the host protein factors associated with the *gp64* sequence shown here have yet to be characterized, they bind to evolutionarily conserved sequence motifs that are recognized by families of eukaryotic transcription factors. Utilization of this apparently "generalist" mechanism of transcriptional activation likely represents a strategy for expressing early genes in the widest possible variety of host cells and thereby adding to the probability of viral expression and replication in cells of a given host species.

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REFERENCES

- Blissard, G. W., P. H. Kogan, R. Wei, and G. F. Rohrmann. 1992. A synthetic early promoter from a baculovirus: roles of the TATA box and conserved start site CAGT sequence in basal levels of transcription. *Virology* **190**:783-793.
- Blissard, G. W., R. L. Quant-Russell, G. F. Rohrmann, and G. S. Beaudreau. 1989. Nucleotide sequence, transcriptional mapping, and temporal expression of the gene encoding P39, a major structural protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*. *Virology* **168**:354-362.
- Blissard, G. W., and G. F. Rohrmann. 1989. Location, sequence, transcriptional mapping, and temporal expression of the *gp64* envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* **170**:537-555.
- Blissard, G. W., and G. F. Rohrmann. 1991. Baculovirus *gp64* gene expression: analysis of sequences modulating early transcription and transactivation by IE1. *J. Virology* **65**:5820-5827.
- Blissard, G. W., and J. R. Wenz. 1992. Baculovirus GP64 envelope glycoprotein is sufficient to mediate pH dependent membrane fusion. *J. Virol.* **66**:6829-6835.
- Bradford, M. E., G. W. Blissard, and G. F. Rohrmann. 1990. Characterization of the infection cycle of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus in *Lymantria dispar* cells. *J. Gen. Virol.* **71**:2841-2846.
- Bucher, P. 1990. Weight matrix descriptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. *J. Mol. Biol.* **212**:563-578.
- Carson, D. D., L. A. Guarino, and M. D. Summers. 1988. Functional mapping of an AcNPV immediate early gene which augments expression of the IE-1 trans-activated 39K gene. *Virology* **162**:444-451.
- Carson, D. D., M. D. Summers, and L. A. Guarino. 1991. Molecular analysis of a baculovirus regulatory gene. *Virology* **182**:279-286.
- Carson, D. D., M. D. Summers, and L. A. Guarino. 1991. Transient expression of the *Autographa californica* nuclear polyhedrosis virus immediate-early gene IE-N is regulated by three viral elements. *J. Virol.* **65**:945-951.
- Carthew, R. W., L. A. Chodosh, and P. A. Sharp. 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. *Cell* **43**:439-448.
- Cherbas, L., and P. Cherbas. 1993. The arthropod initiator: the capsid consensus plays an important role in transcription. *Insect Biochem. Mol. Biol.* **23**:81-90.
- Chisholm, G. E., and D. J. Henner. 1988. Multiple early transcripts and splicing of the *Autographa californica* nuclear polyhedrosis virus IE-1 gene. *J. Virol.* **62**:3193-3200.
- Chodosh, L. A. 1988. Mobility shift DNA-binding assay using gel

- electrophoresis, p. 12.2.1–12.2.10. In F. M. Ausubel et al. (ed.), Current protocols in molecular biology. Wiley, New York.
15. Dang, C. V., C. Dolde, M. L. Gillison, and G. J. Kato. 1992. Discrimination between related DNA sites by a single amino acid residue of myc-related basic-helix-loop-helix proteins. *Proc. Natl. Acad. Sci. USA* **89**:599–602.
 16. Dickson, J. A., and P. D. Friesen. 1991. Identification of upstream promoter elements mediating early transcription from the 35,000-molecular-weight protein gene of *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **65**:4006–4016.
 17. Evans, T., M. Reitman, and G. Felsenfeld. 1988. An erythrocyte-specific DNA-binding factor recognizes a regulatory sequence common to all chicken globin genes. *Proc. Natl. Acad. Sci. USA* **85**:5976–5980.
 18. Fisher, F., and C. R. Goding. 1992. Single amino acid substitutions alter helix-loop-helix protein specificity for bases flanking the core CANNTG motif. *EMBO J.* **11**:4103–4109.
 19. Friesen, P. D., and L. K. Miller. 1985. Temporal regulation of baculovirus RNA: overlapping early and late transcripts. *J. Virol.* **54**:392–400.
 20. Fuchs, L. Y., M. S. Woods, and R. F. Weaver. 1983. Viral transcription during *Autographa californica* nuclear polyhedrosis virus infection: a novel RNA polymerase induced in infected *Spodoptera frugiperda* cells. *J. Virol.* **48**:641–646.
 21. Giacca, M., M. I. Gutierrez, S. Menzo, D. F. F. D'adda, and A. Falaschi. 1992. A human binding site for transcription factor USF-MLTF mimics the negative regulatory element of human immunodeficiency virus type 1. *Virology* **186**:133–147.
 22. Grula, M. A., P. L. Buller, and R. F. Weaver. 1981. Alpha amanitin-resistant viral RNA synthesis in nuclei isolated from nuclear polyhedrosis virus-infected *Heliothis zea* larvae and *Spodoptera frugiperda* cells. *J. Virol.* **38**:916–921.
 23. Guarino, L. A., M. A. Gonzalez, and M. D. Summers. 1986. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **60**:224–229.
 24. Guarino, L. A., and M. Smith. 1992. Regulation of delayed-early gene transcription by dual TATA boxes. *J. Virol.* **66**:3733–3739.
 25. Guarino, L. A., and M. D. Summers. 1986. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. *J. Virol.* **57**:563–571.
 26. Guarino, L. A., and M. D. Summers. 1987. Nucleotide sequence and temporal expression of a baculovirus regulatory gene. *J. Virol.* **61**:2091–2099.
 27. Hoopes, R. R., and G. F. Rohrmann. 1991. *In vitro* transcription of baculovirus immediate early genes: accurate mRNA initiation by nuclear extracts from both insect and human cells. *Proc. Natl. Acad. Sci. USA* **88**:4513–4517.
 28. Huh, N. E., and R. F. Weaver. 1990. Identifying the RNA polymerases that synthesize specific transcripts of the *Autographa californica* nuclear polyhedrosis virus. *J. Gen. Virol.* **71**:195–202.
 29. Hultmark, D., R. Klemenz, and W. Gehring. 1986. Translational and transcriptional control elements in the untranslated leader of the heat shock gene hsp22. *Cell* **44**:429–438.
 30. Klucher, K. M., and D. H. Specter. 1990. The human cytomegalovirus 2.7-kilobase RNA promoter contains a functional binding site for the adenovirus major late transcription factor. *J. Virol.* **64**:4189–4198.
 31. Kovacs, G. R., J. Choi, L. A. Guarino, and M. D. Summers. 1992. Functional dissection of the *Autographa californica* nuclear polyhedrosis virus immediate-early 1 transcriptional regulatory protein. *J. Virol.* **66**:7429–7437.
 32. Kovacs, G. R., L. A. Guarino, and M. D. Summers. 1991. Novel regulatory properties of the IE1 and IE0 transactivators encoded by the baculovirus *Autographa californica* multicapsid nuclear polyhedrosis virus. *J. Virol.* **65**:5281–5288.
 33. Krappa, R., K. A. Behn, F. Jahnel, W. Doerfler, and M. D. Knebel. 1992. Differential factor binding at the promoter of early baculovirus gene PE38 during viral infection: GATA motif is recognized by an insect protein. *J. Virol.* **66**:3494–3503.
 34. Krappa, R., and M. D. Knebel. 1991. Identification of the very early transcribed baculovirus gene PE-38. *J. Virol.* **65**:805–812.
 35. Lee, R. F., M. F. Concino, and R. Weinmann. 1988. Genetic profile of the transcriptional signals from the adenovirus major late promoter. *Virology* **165**:51–56.
 36. Leikina, E., H. O. Onaran, and J. Zimmerberg. 1992. Acidic pH induces fusion of cells infected with baculovirus to form syncytia. *FEBS Lett.* **304**:221–224.
 37. Nissen, M. S., and P. D. Friesen. 1989. Molecular analysis of the transcriptional regulatory region of an early baculovirus gene. *J. Virol.* **63**:493–503.
 38. Omichinski, J. G., G. M. Clore, O. Schaad, G. Felsenfeld, C. Trainor, E. Appella, S. J. Stahl, and A. M. Gronenborn. 1993. NMR structure of a specific DNA complex of zinc-containing DNA binding domain of GATA-1. *Science* **261**:438–446.
 39. Orkin, S. H. 1992. GATA-binding transcription factors in hematopoietic cells. *Blood* **80**:575–581.
 40. Passarelli, A. L., and L. K. Miller. 1993. Three baculovirus genes involved in late and very late gene expression: *ie-1*, *ie-n*, and *lef-2*. *J. Virol.* **67**:2149–2158.
 41. Ray, F. A., and J. A. Nickoloff. 1992. Site-specific mutagenesis of almost any plasmid using a PCR-based version of unique site elimination. *Biotechniques* **13**:342–348.
 42. Reach, M., L. E. Babiss, and C. S. H. Young. 1990. The upstream factor-binding site is not essential for activation of transcription from the adenovirus major late promoter. *J. Virol.* **64**:5851–5860.
 43. Rice, W. C., and L. K. Miller. 1987. Baculovirus transcription in the presence of inhibitors and in nonpermissive *Drosophila* cells. *Virus Res.* **6**:155–172.
 44. Rohel, D. Z., and P. Faulkner. 1984. Time course analysis and mapping of *Autographa californica* nuclear polyhedrosis virus transcripts. *J. Virol.* **50**:739–747.
 45. Rohrmann, G. F. 1992. Baculovirus structural proteins. *J. Gen. Virol.* **73**:749–761.
 46. Sawadogo, M. 1988. Multiple forms of the human gene-specific transcription factor USF: II. DNA binding properties and transcriptional activity of the purified HeLa USF. *J. Biol. Chem.* **263**:11994–12001.
 47. Sawadogo, M., and R. G. Roeder. 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. *Cell* **43**:165–176.
 48. Sawadogo, M., D. M. E. Van, P. D. Gregor, and R. G. Roeder. 1988. Multiple forms of the human gene-specific transcription factor USF. I. Complete purification and identification of USF from HeLa cell nuclei. *J. Biol. Chem.* **263**:11985–11993.
 49. Skeiky, Y. A. W., and K. Iatrou. 1991. Synergistic interactions of silkworm chorion promoter-binding factors. *Mol. Cell. Biol.* **11**:1954–1964.
 50. Theilmann, D. A., and S. Stewart. 1991. Identification and characterization of the IE-1 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* **180**:492–508.
 51. Theilmann, D. A., and S. Stewart. 1992. Molecular analysis of the transactivating IE-2 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* **187**:84–96.
 52. Theilmann, D. A., and S. Stewart. 1992. Tandemly repeated sequence at the 3' end of the IE-2 gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus is an enhancer element. *Virology* **187**:97–106.
 53. Volkman, L. E. 1986. The 64K envelope protein of budded *Autographa californica* nuclear polyhedrosis virus. *Curr. Top. Microbiol. Immunol.* **131**:103–118.
 54. Volkman, L. E., and P. A. Goldsmith. 1985. Mechanism of neutralization of budded *Autographa californica* nuclear polyhedrosis virus by a monoclonal antibody: inhibition of entry by adsorptive endocytosis. *Virology* **143**:185–195.
 55. Volkman, L. E., P. A. Goldsmith, R. T. Hess, and P. Faulkner. 1984. Neutralization of budded *Autographa californica* NPV by a monoclonal antibody: identification of the target antigen. *Virology* **133**:354–362.
 56. Whitford, M., S. Stewart, J. Kuzio, and P. Faulkner. 1989. Identification and sequence analysis of a gene encoding gp67 an abundant envelope glycoprotein of the baculovirus *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **63**:1393–1399.