Characterization of the Human Cytomegalovirus UL75 (Glycoprotein H) Late Gene Promoter

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Glycoprotein H (gH, UL75) of human cytomegalovirus (HCMV) is an essential envelope glycoprotein that functions in viral entry and the activation of gene expression. To understand the regulation of this important viral gene, the promoter of the UL75 late gene was characterized in HCMV-infected cells at the late stages of viral infection. Primer extension analysis revealed a single major start site located 26 bp downstream of a putative TATA element. Deletion analysis showed the presence of a dominant activation domain from +14 to +35 that masked regulatory sequences upstream of the TATA element. Mutational analysis demonstrated that a PEA3-like element in this downstream domain was important for promoter activation. In addition, gel shift analysis revealed direct protein binding to the PEA3-like element. Together, these studies reveal that the gH promoter is regulated in a complex manner with sequences both upstream and downstream of the cap site influencing promoter activation. © 2002 Elsevier Science (USA)

Key Words: human cytomegalovirus; glycoprotein H; late gene expression; transcriptional regulation.

INTRODUCTION

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that infects 40–60% of the population in the United States (reviewed in Mocarski and Courcelle, 2001). Disease caused by HCMV primarily occurs in the immunocompromised and is an increasing problem with the advent of AIDS and the growing numbers of organ and bone marrow transplant recipients. Congenital HCMV infections can also result in severe sequelae in the infected fetus (reviewed in Pass, 2001). Infection by HCMV is initiated by virus binding to a cellular receptor(s) via envelope glycoproteins, followed by fusion of the viral and cellular envelopes (reviewed in Mocarski and Courcelle, 2001). Glycoprotein H (gH, UL75) is an essential viral envelope glycoprotein (Hobom *et al.,* 2000) that is thought to play a key role in virus–cell fusion (Baldwin *et al.,* 2000; Milne *et al.,* 1998). In addition, gH is involved in triggering signal transduction cascades that may assist in the initiation of viral gene expression (Yurochko *et al.,* 1997). The subsequent expression of viral mRNAs occurs in a coordinated cascade consisting of immediate early (IE), early, and late genes (reviewed in Mocarski and Courcelle, 2001). The IE proteins are expressed first in permissively infected cells and are necessary to trigger subsequent viral early gene expression. The early proteins include those necessary for viral DNA replication. Late genes are subsequently expressed after

viral DNA replication and typically encode viral structural proteins, including gH.

Numerous studies have addressed the regulation of viral early gene expression (reviewed in Fortunato and Spector, 1999; Mocarski and Courcelle, 2001). However, only two HCMV late promoters, UL94 and UL99, have been studied in detail (Depto and Stenberg, 1992; Kerry *et al.,* 1997; Kohler *et al.,* 1994; Wing *et al.,* 1998). These analyses demonstrated that little sequence upstream of the TATA element was required to activate HCMV late promoters. For example, UL99 sequences from −40 to +106 were sufficient to activate this promoter with wild-type kinetics in the context of the viral genome (Kohler *et al.,* 1994). This finding is consistent with the analysis of late gene regulation in herpes simplex virus (reviewed in Roizman and Knipe, 2001), suggesting that such simple promoter regions may be a common feature of herpesvirus late gene regulation. For example, sequences from −50 to +33 of the UL38 late gene of herpes simplex virus were found to be sufficient for promoter activation (Guzowski and Wagner, 1993). However, additional elements have also been determined to influence HCMV late gene expression. Analyses of the HCMV UL99 gene revealed that expression is regulated posttranscriptionally, via secondary structure in the 5′ untranslated region of the UL99 mRNA (Kerry *et al.,* 1997), demonstrating that more than one level of regulation may be involved in controlling the appearance of late gene products. Analysis of the HCMV UL94 promoter in transient transfection assays demonstrated the presence of a dominant downstream activation domain located from +1 to +48 (Wing *et al.,* 1998). Upon deletion of this region, p53 and IE86 binding sites located upstream of the TATA element were

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found to repress UL94 promoter function. This study therefore suggests that sequences upstream of the TATA element and downstream of the cap site can cooperatively regulate the expression of the UL94 late promoter (Wing *et al.,* 1998).

HCMV gH is part of a three-protein complex known as gCIII, including gL (UL115) and gO (UL74) (Huber and Compton, 1997, 1999). The gH glycoprotein is conserved throughout known herpesviruses (Cranage *et al.,* 1988) and recent studies using transposon insertion mutagenesis strongly suggest that gH is essential for HCMV replication (Hobom *et al.,* 2000). Antibodies to gH have been found to block the ability of the viral membrane to fuse with the membrane of the host cell, implicating gH in virus-cell fusion (Keay and Baldwin, 1991). In addition, gH is a major target for neutralizing antibodies generated during the natural course of infection (Urban *et al.,* 1996). Direct stimulation of the gH receptor by anti-idiotypic antibodies also results in the up-regulation of the transcription factors Sp1 and NF-κB in fibroblasts, suggesting that gH may be involved in the initiation of viral gene expression (Yurochko *et al.,* 1997). Together, these findings indicate that gH plays a crucial role in the viral replicative cycle and is an important target for the host immune response.

In the present study, we characterized the promoter of the HCMV gH late gene, UL75, in HCMV-infected cells at the late stages of viral infection. Primer extension analysis was used to map the 5′ end of the mRNA. Deletion analysis revealed the presence of a dominant activation domain downstream of the mRNA start site that masks both activation and repression domains within upstream promoter sequences. The downstream activation domain contains a consensus binding site for the cellular transcription factor PEA3. Mutation of this site resulted in the abrogation of promoter activity and gel shift analysis revealed a protein present in cells at late times of infection that specifically bound to the PEA3-like element. Intriguingly, deletion of this downstream sequence resulted in a construct with wild-type promoter activity, suggesting that this region may also contain a binding site for a repressor. Together, our results demonstrate a complex mechanism of regulation of this HCMV late gene, with the PEA3-like element playing a critical role in UL75 promoter activation.

RESULTS

Mapping the UL75 transcriptional initiation site

Previous analysis of HCMV late promoters demonstrated that sequences located downstream of the cap site had significant effects on promoter activity (Depto and Stenberg, 1992; Kerry *et al.,* 1997; Kohler *et al.,* 1994; Wing *et al.,* 1998). To assess if this was also the case for the UL75 promoter, we first localized the site of transcriptional initiation by primer extension analysis (Fig. 1A). An

oligonucleotide primer was designed complementary to sequences 108 bp downstream of the putative TATA element of the UL75 promoter. Primer extension of RNA isolated from human fibroblast cells (HFF) at the late stages of infection showed a prominent intense band of 82 bp in length (Fig. 1) that was not present in an identically treated sample from uninfected HFF cells. This experiment therefore identifies the major transcriptional initiation site as 26 bp downstream of the TATA element (Fig. 1B). Some additional, less intense bands were also detected that likely represent premature termination of the primer extension due to secondary structure within the GC rich 5′ end of the UL75 mRNA, or products of minor transcription start sites.

Deletion analysis of the UL75 promoter

In order to assess the sequences required for promoter activation, we cloned UL75 promoter sequences from −453 to +35 into the reporter vector pSV0CAT to generate pUL75CAT. Sequence analysis using the DNAsis program demonstrated that this region contains a number of elements that may be involved in transcriptional activation (Fig. 1B). In addition, the UL75 promoter was found to contain a region from +1 to +6 with some sequence similarity to a DAS element (Guzowski *et al.,* 1994; Guzowski and Wagner, 1993; Petroski *et al.,* 2001). This element was originally identified downstream of the cap site of the herpes simplex virus UL38 late promoter, and has been shown to be required for activated transcription of this promoter (Guzowski and Wagner, 1993). To determine sequences necessary for UL75 promoter activation, a series of deletion constructs were generated removing sequences both upstream and downstream of the transcriptional initiation site (Fig. 2A). The deletion constructs were then transfected into HFF cells and evaluated for their response to viral infection. These experiments show that deletion of sequences from −453 to −38 in the presence of promoter elements from +14 to +35 had no significant effect on UL75 promoter activity (Fig. 2B), suggesting that sequences upstream of the TATA element are not essential for UL75 promoter activation.

Analysis of the HCMV UL94 late promoter demonstrated that downstream promoter elements worked in combination with upstream elements to regulate transcription (Wing *et al.,* 1998). To assess if a similar mechanism was involved in the regulation of UL75 promoter activity, sequences from +14 to +35 were deleted and assessed for the effect on promoter activation. In the context of UL75 promoter sequences from −453, deletion of the region from +14 to +35 resulted in no significant change in promoter activity (Figs. 2B and 3). In contrast, combining the deletion from +14 to +35 with deletion of sequences from −453 to −245 resulted in a significant increase in activity, to approximately twice
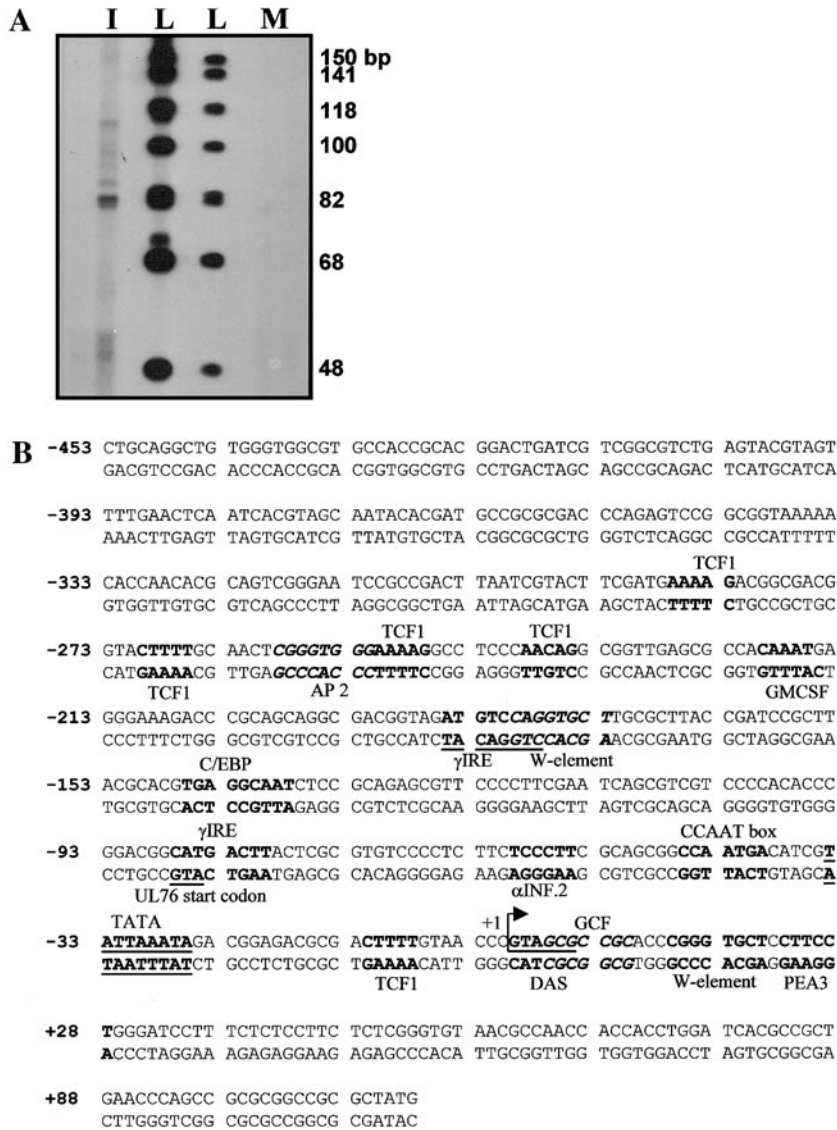
FIG. 1. (A) Primer extension analysis of the UL75 promoter. Total cellular RNA from infected (I) and mock-infected (M) HFFs was subjected to primer extension analysis to map the UL75 transcriptional start site. The size of the extension products was assessed by comparison with a radiolabeled DNA ladder (L). The sizes of the ladder DNA are indicated on the right. (B) Sequence of the UL75 promoter region. Consensus transcription factor binding elements are indicated in bold. In cases where sequences overlap, one of the sequences is underlined and the other italicized. The location of the label denotes the orientation of the consensus element. The UL75 transcription start site (+1), TATA box, and the UL76 start codon are also indicated.

that of the intact UL75 promoter region. This finding suggests that the sequences from −453 to −245 may contain a repressor element. Alternatively, this effect may be nonspecific, as similar increases in promoter activity have been observed in analyses of several other HCMV promoter regions (J. A. Kerry, unpublished observations).

Further deletion of sequences from −245 to −117 in the context of the +14 to +35 deletion resulted in a significant reduction in promoter activity, suggesting that sequence elements between −245 and −117 are involved in the activation of the UL75 promoter function (Fig. 2B). This effect is observed only in the absence of the +14 to +35 sequence, implying that this region functions as a dominant downstream domain, similar to

that observed in the UL94 promoter. Additional deletion of the −117 to −81 sequence resulted in a significant 2.5-fold increase in activity, suggesting the presence of a repressor element between −117 and −81. Subsequent deletion of sequences from −81 to −38 in the absence of the region from +14 to +35 results in a decrease in promoter activity to a level similar to that of the wild-type promoter construct, indicating the presence of another activation domain between −81 and −38. Together, these data show that in the absence of sequences from +14 to +35, elements upstream of the TATA box can function to both positively and negatively regulate UL75 promoter activity. In addition, they suggest that the sequences from +14 to +35 may act as a dominant do-
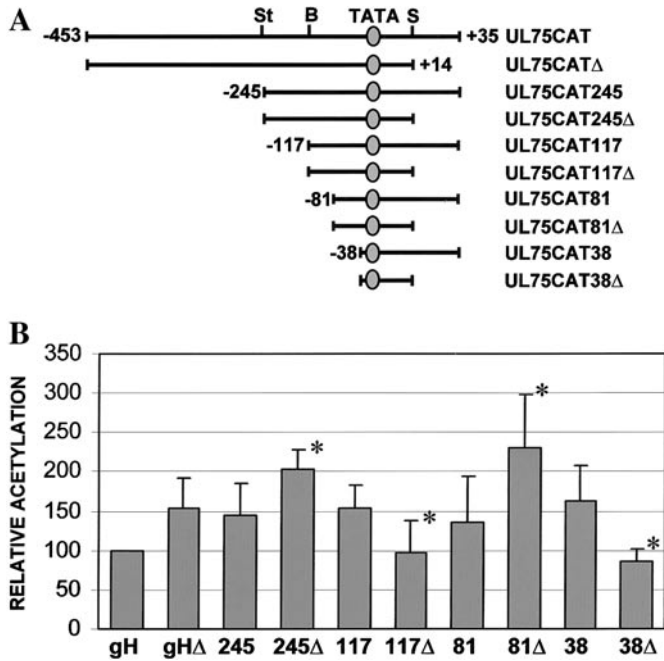
FIG. 2. (A) Schematic diagram of the UL75 promoter. Deletions generated within the promoter region are indicated (St, *Stu*I; B, *Bst*BI; S, *Sma*I). (B, C) Activation of UL75 promoter deletions by viral infection. (B) Constructs containing deletions of the UL75 promoter in the presence or absence (Δ) of sequences from +15 to +35 were assessed by transfection–superinfection analysis as described under Materials and Methods. Activity is expressed relative to the wild-type promoter at 100%. The average (+ standard deviation) from at least two experiments performed in duplicate is shown. gH, pUL75CAT; gHΔ, pUL75CATΔ; 245, pUL75CAT245; 245Δ, pUL75CAT245Δ; 117, pUL75CAT117; 117Δ, pUL75CAT117Δ; 81, pUL75CAT81; 81Δ, pUL75CAT81Δ; 38, pULCAT38; 38Δ, pUL75CAT38Δ. *Significant differences (*P* ≤ 0.05), based on Student's *t* tests performed on the analysis of the UL75 mutants in the absence of sequences from +15 to +35.

main. Further, sequences from −38 to +14 are sufficient to activate the UL75 promoter in the context of transient assays.

Mutational analysis of the UL75 promoter

Deletion analysis suggested that sequences from +14 to +35 might play an important role in the regulation of UL75 promoter function. This region contains two consensus enhancer elements, a W-element (Cogswell *et al.,* 1990, 1991) and a putative PEA3 binding site (Wasylyk *et al.,* 1990; Xin *et al.,* 1992). To assess the role of these sequences in UL75 promoter activation, nucleotide substitutions were generated in the context of the full-length promoter (Fig. 3). The UL75 promoter constructs containing the mutated W-element or PEA3-like element were transfected into fibroblasts and evaluated for their response to viral infection (Fig. 3). These analyses demonstrated that mutation of the W-element had no effect on UL75 promoter activity, indicating that this element is not required for promoter function. In contrast, mutation of the PEA3-like element resulted in a six- to sevenfold drop

in UL75 promoter activity. Similar effects were observed when the PEA3 site was mutated in the context of the minimal promoter construct from −38 to +35 (data not shown). These findings suggest that the PEA3-like element may play a critical role in the regulation of UL75 promoter function. This was somewhat surprising, as deletion of this region had no significant effect on promoter activity in the context of the minimal promoter construct. To determine if other sequence elements within the minimal promoter region from −38 to +14 could also be involved in UL75 regulation, a series of scanning mutants between −25 and +9 were generated, including a mutation of the putative DAS element. Analysis of these mutants demonstrated that no individual sequence element within the minimal promoter had a significant impact on promoter activation (data not shown). Thus, the PEA3-like element appears to be the primary functional element within this region.

Protein binding analysis of the UL75 promoter

Our analysis of the UL75 promoter revealed that a PEA3-like element downstream of the transcriptional initiation site was important for transcriptional activation. We therefore assessed the ability of proteins present within both infected and uninfected cells to bind to this sequence element (Fig. 4). These studies demonstrate the presence of a protein within nuclear extracts isolated from infected cells at late times after viral infection that bound specifically to the PEA3-like element (Band P).
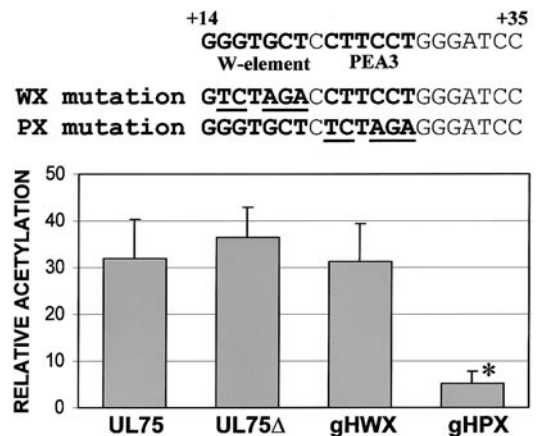

FIG. 3. (Top) Sequence from +14 to +35 of the UL75 promoter elements. The consensus binding sites for the W-element and PEA3 proteins are indicated. Mutations of these elements are shown underneath the sequence with the mutated nucleotides underlined. (Bottom) Activation of UL75 promoter mutations by viral infection. Constructs containing mutations of the UL75 promoter in the context of the full-length promoter were assessed by transfection–superinfection analysis as described under Materials and Methods. Activity is expressed relative to the wild-type promoter at 100%. The average (+ standard deviation) from at least two experiments performed in duplicate is shown. UL75, pUL75CAT; UL75Δ, pUL75CATΔ; UL75WX, pUL75CATWX; UL75PX, pUL75CATPX. *P* ≤ 0.01 (Student's *t* test).
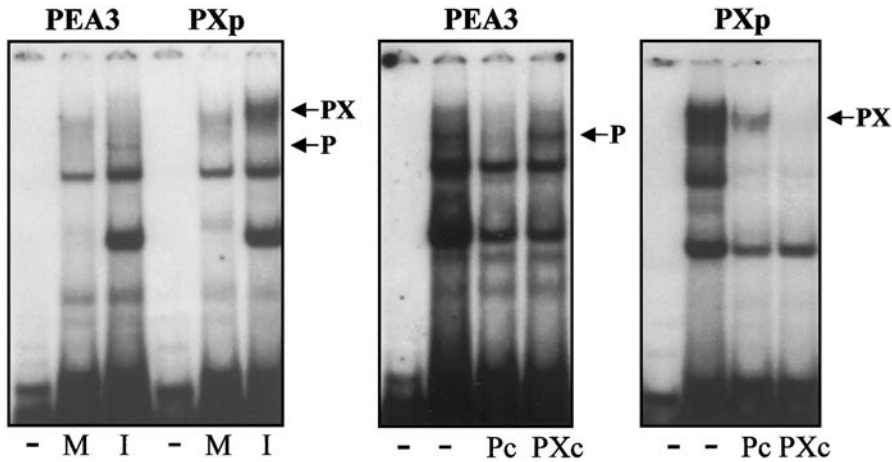
FIG. 4. Gel-shift analysis of proteins binding to the UL75 promoter region. Oligonucleotide probes containing either the wild-type PEA3-like element (PEA3) or the PX mutation (PXp) were assessed by gel shift analysis using nuclear extracts isolated from mock-infected HFFs (M) or cells infected for 72 h (I). Competition analysis was carried out with 100-fold excess of either the wild-type sequence (Pc) or the mutated probe DNA (PXc). Two specific bands, P and PX, associated with the PEA3 probe or mutated probe, respectively, are indicated by the arrows.

The two additional bands detected in the gel shift analysis represent nonspecific binding products. Competition analysis revealed that Band P could be competed with 100-fold excess of the wild-type probe, but not with DNA containing a mutation in the PEA3-like element. In addition, we were unable to detect this band in nuclear extracts isolated from uninfected cells. Thus, it is likely that this protein is involved in the activation of the UL75 promoter. PEA-3 is a member of the ets family of transcription factors, and actually consists of a family of related proteins (de Launoit et al., 1997). To further characterize the specific factor binding to the PEA3-like element, we performed competition analysis using oligonucleotides containing consensus ets and PEA3 binding elements, as well as oligonucleotides containing mutations of the consensus binding sites. These studies demonstrated that oligonucleotides containing either the ets or PEA3 consensus binding sites were both capable of competing efficiently for binding to the UL75 PEA3-like element (data not shown). In contrast, oligonucleotides containing the mutated ets and PEA3 elements were unable to compete for binding to the UL75 PEA3-like element, implying that the protein binding to this element is closely related to the ets/PEA3 family of transcription factors, or is a unique protein with a similar binding site. Interestingly, we also detected a different protein–DNA complex within infected cell nuclear extracts when we used the probe containing the mutated PEA3-like element (Band PX). This protein–DNA complex was efficiently competed with the PX probe, but only poorly competed with 100-fold excess of the wild-type DNA fragment. This finding suggests that proteins present within the infected cell at late times may be able to bind to the DNA in the absence of the PEA3-like element that potentially play a role in transcriptional regulation via this region.

DISCUSSION

Gene expression in HCMV-infected cells is a complex process that is controlled by both viral and cellular proteins (reviewed in Fortunato and Spector, 1999; Mocarski and Courcelle, 2001). The goal of this study was to characterize sequences required for activation of the gH (UL75) late promoter. These analyses demonstrated that sequences downstream of and including the TATA element are primarily responsible for activation of the UL75 promoter in HCMV-infected cells at the late stages of infection. Specifically, sequences from -38 to $+35$ were found to be sufficient for activation. This finding is similar to the analysis of the UL94 and UL99 HCMV late promoters, as well as herpes simplex virus late promoters. In these investigations, it was determined that sequences upstream of the TATA element were not required for late promoter activation both in transient transfection assays and in the context of the viral genome (Depto and Stenberg, 1992; Kohler et al., 1994; Roizman and Knipe, 2001; Wing et al., 1998). However, in the analyses of HCMV late gene expression, no specific element required for activation of the HCMV late promoters had been identified. In the present study, we have characterized a specific element that significantly impacts on the activation of the UL75 late promoter. Our studies identified a PEA3-like element located at $+23$ to $+28$ within the minimal UL75 promoter region that significantly impacted on UL75 promoter activity. In support of a role for this element in UL75 promoter activation, we identified a protein present within late infected cells that specifically bound to the wild-type PEA3-like element. Competition analysis demonstrated that the protein binding to this element is likely closely related to the ets/PEA3-family of transcription factors.

Analysis of the HCMV UL99 late promoter had re-

vealed that expression of this gene is regulated at the level of translation via a stem–loop structure within the 5′-untranslated region (Kerry et al., 1997). However, the UL75 downstream sequences, while relatively GC-rich, did not contain sequences that would permit the formation of a stable stem–loop structure. Therefore, it is most likely that UL75 regulation via the PEA3-like site occurs at the level of transcription. Our previous analyses of the UL99 promoter determined that regions critical in transcriptional activation identified using transient assays are also involved in the regulation of gene expression in the context of the viral genome (Depto and Stenberg, 1992; Kerry et al., 1997; Kohler et al., 1994), demonstrating that transient transfection analysis is a valid method to identify elements involved in the regulation of HCMV late gene expression. To confirm the role of the PEA3-like element in UL75 promoter regulation, we are currently inserting the UL75 promoter derivatives into the viral genome to assess the effects of the promoter mutations in the context of a natural infection.

Our analysis of the minimal UL75 promoter region revealed that deletion from +14 to +35 resulted in a promoter construct with activity similar to that of the wild-type promoter region. Within these sequences, scanning mutational analysis did not reveal any single region that significantly impacted on UL75 promoter activity, implying that the TATA element was sufficient for UL75 promoter activation by viral proteins. This was somewhat surprising, as mutational analysis clearly demonstrated that the PEA3-like element is required for UL75 promoter activation. In the context of the mutated PEA3-like element, we hypothesize that the mutated sequences may bind a protein that blocks activation of the UL75 promoter. This hypothesis is supported by the direct binding analysis using the PX probe (Fig. 4) that revealed a specific protein binding to the PX region in infected cell nuclear extracts. One possibility is that the specific mutation introduced in this region resulted in the formation of a binding site for a different transcription factor. Screening of the mutated sequence using the TRANSFAC database revealed that this mutation resulted in a partial binding site for thyroid transcription factor 1 (Wingender et al., 2000). This factor is expressed only in thyroid, lungs, and the developing brain (Guazzi et al., 1990) and thus is unlikely to play a role in regulating the UL75 promoter in fibroblasts. Alternatively, the factor binding to the PX region may have a specific function in UL75 promoter regulation. For example, it is possible that this region of the promoter may bind two proteins, the PEA3-like factor and a putative repressor protein. In the presence of the wild-type sequence element, proteins binding to the PEA3-like element would prevent binding of this novel protein to the UL75 promoter. Similarly, deletion of this region would result in an inability of the repressor to bind, resulting in a loss of repression of the UL75 promoter. Identification of the proteins that bind to

both the wild-type and the mutated sequences will enable us to characterize the precise role of these proteins in the regulation of the UL75 promoter via the PEA3-like element.

Our deletion analysis suggested that the region from +14 to +35 functioned as a dominant downstream control element that masks the effect of potential activation and repression domain elements upstream of the TATA box. A similar phenomenon was observed with the UL94 late promoter (Depto and Stenberg, 1992; Kohler et al., 1994; Wing et al., 1998). One likely reason for multiple control elements within the promoters of HCMV genes is related to the variety of cell types that HCMV infects in vivo. For example, the PEA3 protein is expressed in fibroblasts and epithelial cells, but not in cells of the hematopoietic system (Xin et al., 1992). As HCMV infects both hematopoietic progenitor cells and macrophages (Mocarski and Courcelle, 2001), alternate mechanisms for activation of the UL75 promoter may be required for efficient expression of this essential viral gene. In future studies, we will directly assess UL75 promoter activation in other permissive cell types in order to test this hypothesis. In summary, these studies demonstrate that HCMV late promoter activation is a complex process in which both activators and repressors combine to carefully control the expression of these essential viral genes.

MATERIALS AND METHODS

Plasmids

The UL75 promoter region from nucleotide 110208 to 110694 (Chee et al., 1990) was cloned into a modified pUC12 vector (pUCHH) with a HindIII site at each end of the multiple cloning site (MCS). The 488-bp UL75 promoter sequences were cloned into the CAT reporter vector pSV0CAT (Depto and Stenberg, 1989) to generate pUL75 CAT. The UL75 promoter deletion mutants pUL75CAT245 and pUL75CAT117 were generated by digestion with either StuI (−245) or BstBI (−117) and a PstI site located in the pUCHH MCS, followed by religation of the vector DNA. The resultant truncated promoter fragments were subcloned as HindIII fragments into pSV0CAT. Additional deletions were generated using a PCR mutagenesis strategy (Chau et al., 1999) designed to introduce HindIII sites at −81 (pUL75CAT81: primers 81f 5′-GCA**AAG**CTTACTCGCGTGTCCCCTCTTCTC and 81r 5′-GAGAAGAGGGGACACGCGAGTAAG**CTT**TGC; mutated sequences indicated in bold) and −38 (pUL75CAT38: primers 38f 5′-CCCTTCGCAGCGGCCAA**GCTT**ATCGTATTAAAT-AGACG and 38r 5′-CGTCTATTTAATACGATAA**GCTT**GGC-CGCTGCGAAGGG) in the UL75 promoter. The resultant truncated HindIII fragments were consequently subcloned into the CAT reporter vector. Deletion of sequences from +15 to +35 (pUL75CATΔ) was accomplished by digestion with a SmaI site that cuts at +14 in the UL75 promoter and at the 3′ end of the promoter.

Promoter variants containing mutations of the W-element (pUL75CATWX) and PEA3 site (pUL75CATPX) were generated by cloning oligonucleotide fragments containing sequences from +15 to +35 with the mutated element into the *Sma*I site. The primers used are shown with the mutated region indicated in bold: WXf 5′, CCGGT**CTA-GA**CTTCCTGGGATCC; WXr 5′, CCGGGGATCCCAGGA-AG**TCTAGA**; PXSf 5′, CCGGTGCTC**TCTAGA**GGGATCC; PXSr 5′, CCGGGGATCCC**TCT**A**GA**GAGCAC.

Cells and transfection assays

Primary HFFs were transfected with 10 μg of DNA by the DEAE-dextran method as previously described (Depto and Stenberg, 1989). Twenty-four hours after transfection the cells were infected with HCMV strain Towne. Cells were harvested 72 h after infection and assessed for CAT enzyme activity (Depto and Stenberg, 1989).

Primer extension analysis

Total cellular RNA was isolated from mock-infected and HFF cells infected for 72 h to allow for late gene expression using the RNeasy system as described in the manufacturer's protocol (Qiagen, Chatsworth, CA). The RNA samples were subjected to primer extension analysis as previously described (Triezenberg, 1992) using the oligo UL75primerext 5′-GTGATCCAGGTGGTGGTTG-GCGTTAC.

Gel shift assays

Nuclear extracts from HFF cells infected for 72 h or mock-infected were isolated as previously described (Kerry *et al.,* 1994). Gel shift analysis was also performed as previously described except that 0.2 to 1 μg of the nonspecific competitor poly(dI-dC) was used (Chau *et al.,* 1999). Probes containing wild-type sequences were generated by annealing the oligonucleotides Pf 5′-GGT-GCTCCTTCCTGGGATCC and Pr 5′-GGATCCCAGGAAG-GAGCACC. The PX probe was generated by annealing PXf 5′-GGTGCTC**TCTAGA**GGGATCC and PXr 5′-GGA-TCCC**TCTAGA**GAGCACC. Oligonucleotides containing consensus ets and PEA3 sites and their mutated derivatives used in competition experiments were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

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