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The Acidic Domain of pUL37x1 and gpUL37 Plays a Key Role in Transactivation of HCMV DNA Replication Gene Promoter Constructions

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Transient complementation of human cytomegalovirus (HCMV) *ori*Lyt DNA replication in permissive human diploid cells expressing replication genes under native promoters requires its UL36-38 gene products. Two of the immediate early (IE) proteins encoded by this locus, pUL37x1 and, to a lesser extent, gpUL37, activated expression of HCMV early gene promoter constructions. The other IE protein encoded by the UL36-38 locus, pUL36, and the early product, pUL38, did not transactivate the HCMV early promoter constructions under similar conditions. The acidic domain, common to both pUL37x1 and gpUL37, is required for activation of HCMV early promoter constructions. Conversely, gpUL37 sequences downstream of amino acid 199 are not required for transactivation of viral early promoters. Taken together, these results suggest that the requirement for UL36-38 products for HCMV DNA replication results, at least in part, from the requirement of the transactivation of HCMV early DNA replication promoters by pUL37x1 and, to a lesser extent, by gpUL37 and that the acidic domain is critical for this activity.

INTRODUCTION

Although similar to alphaherpesvirus DNA replication, human cytomegalovirus (HCMV) *ori*Lyt DNA replication appears to be more complex as its origin spans 2.4 kbp and requires 11 distinct genetic loci as determined in transient assays (reviewed in Anders and McCue, 1996). Among the required loci are 3 immediate early (IE) loci, which do not encode replication fork proteins and include the UL36-38 locus (Pari and Anders, 1993; Pari *et al.,* 1993).

The UL36-38 locus is complex in that it encodes at least four transcripts; the UL36, UL37, and UL37x1/38 RNAs are IE in temporal expression, whereas the UL38 RNA is an early product (Fig. 1A, Wilkinson *et al.,* 1984; Kouzarides *et al.,* 1988; Tenney and Colberg-Poley, 1991a,b; reviewed in Colberg-Poley, 1996). Antisense experiments are consistent with the requirement for UL36 and UL37 gene products for HCMV growth and DNA replication (Smith and Pari, 1995; Pari *et al.,* 1995). The dependence upon UL36-38 products for HCMV *ori*Lyt DNA replication is lost by the presence of UL69 in Vero cells but not in permissive human diploid fibroblasts (HFF) (Sarisky and Hayward, 1996).

The products of the IE transcripts include the UL36 protein (pUL36), a member of the US22 family (Chee *et al.,* 1990), the UL37 exon 1 protein (pUL37x1), and the UL37 N-glycoprotein (gpUL37). pUL36 does not share its sequences with any of the other UL36-38 products. In contrast, gpUL37 and pUL37x1 proteins are encoded by partially overlapping transcripts and share the amino-terminal 162 amino acids (aa) of their respective open reading frames (ORF, Fig. 1B). Their common features include a hydrophobic signal sequence (aa 1–22) and a strongly charged acidic domain (aa 81–108). Unique to gpUL37 are 17 N-glycosylation sites, a basic domain, a transmembrane/anchor sequence, and a cytosolic tail (Kouzarides *et al.,* 1988; Chee *et al.,* 1990). gpUL37 is synthesized in the HCMV-infected cell as a type I membrane-bound protein, traffics through the endoplasmic reticulum and Golgi apparatus, and is modified by N-glycosylation within 2 h of synthesis (Al-Barazi and Colberg-Poley, 1996). It does not detectably colocalize with the HCMV major IE protein 1 (IE1) (Zhang *et al.,* 1996).

The aforementioned requirement for UL36-38 products for HCMV *ori*Lyt DNA replication may result from their regulatory activities. Similarly to most HCMV IE proteins, pUL36, pUL37x1, and gpUL37 have nuclear regulatory activity as demonstrated by regulation of a human *heat shock protein* 70 (*hsp*70) promoter construction (Colberg-Poley *et al.,* 1992; Tenney *et al.,* 1993; Zhang *et al.,* 1996). Consistent with these findings, a genomic construction spanning the HCMV UL33-38 region was found previously to transactivate HCMV early promoter construc-

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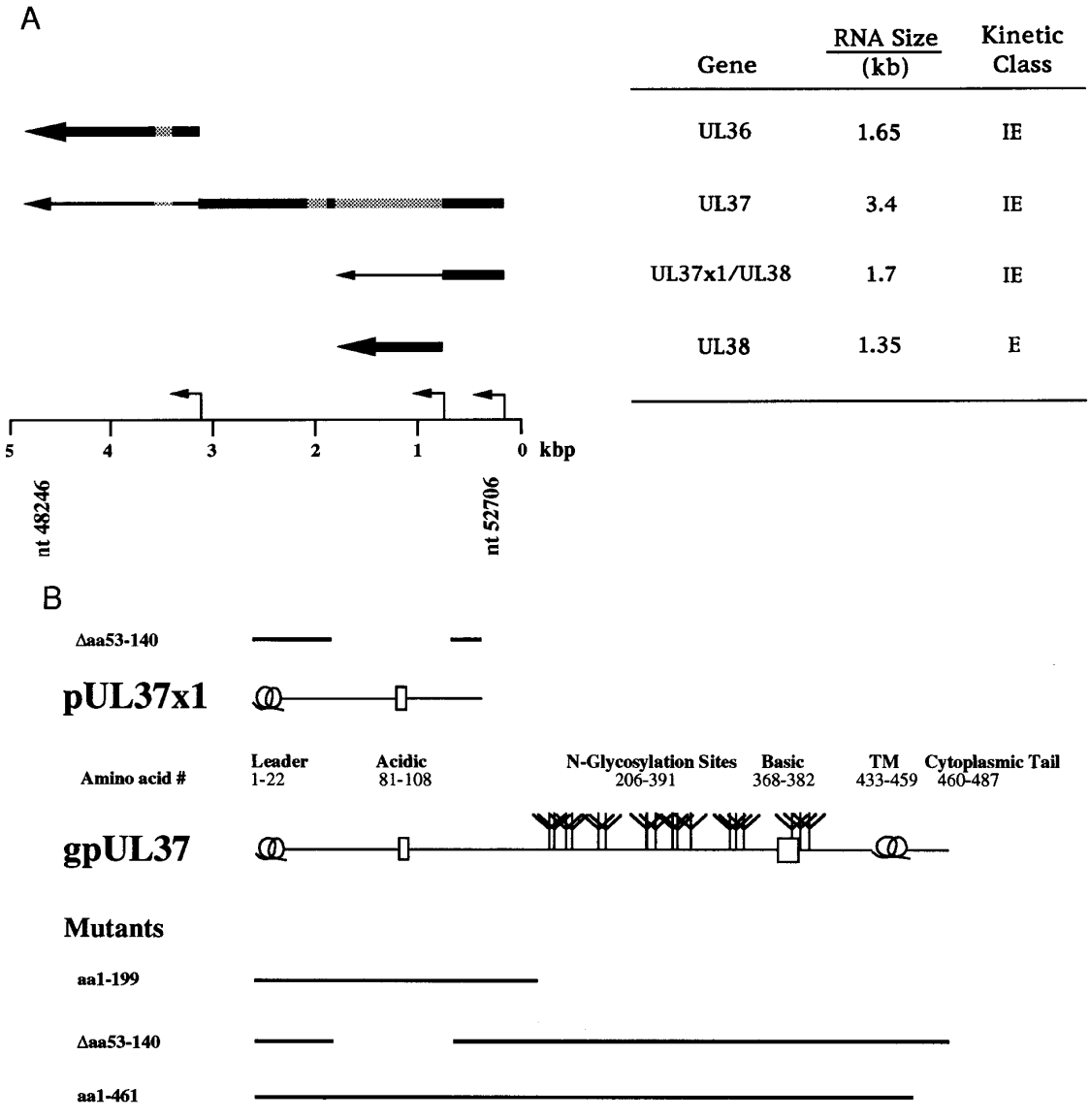

FIG. 1. The products of the HCMV UL36-38 locus. (A) The UL36-38 transcripts. The UL36-38 locus spans nt 52,706 to 48,246 of the HCMV genome. The alternatively spliced transcripts encoded by the region are shown as are their respective sizes and kinetic class. The bent arrows represent the promoters within the locus and the exons (filled boxes) and introns (strippled boxes) are indicated. (B) The wild-type and mutant pUL37x1 and gpUL37 proteins. Shown are the amino-terminal sequences (aa 1–162) common to both pUL37x1 and gpUL37. These include the hydrophobic signal sequence (aa 1–22, coil) and acidic domain (aa 81–108, open box). pUL37x1 as one additional amino acid. Unique to gpUL37 (aa 163–487) are a large N-glycosylation domain (aa 206–391, branches), a transmembrane (aa 433–459, coil), and cytoplasmic domains (aa 460–487). The mutants lacking the acidic domain (pUL37x1 Δaa 53–140 and gpUL37 Δaa 53–140) are shown as are the gpUL37 truncation mutants, gpUL37 aa 1–199 and gpUL37 aa 1–461.

tions (Iskenderian *et al.,* 1996). In these experiments, we determined which of the UL36-38 IE and early proteins transactivate HCMV early DNA replication gene promoter constructions and which domain in the regulatory proteins is required for this stimulation.

RESULTS

Our previous results indicate that the UL33-38 region, encoded by pZP8, contributes significantly to the regulation of HCMV early gene transcription (Iskenderian *et al.,* 1996). To determine whether the UL36-38 IE region contained within this genomic construction encodes the proteins required for this regulation, we tested deletion mutants of the UL33-38 genomic clone for regulation of the HCMV UL54 promoter (Fig. 2). As found previously, the combination of TRS1, pUL84, IE1, IE2, and UL112-113, referred to hereafter as All, with the genomic clone UL33-38 (All (UL33-38)) transactivated the UL54 promoter. This value of transactivation was taken as 100%. Cells transfected with deletion of the genomic UL33 locus (All (UL34-38)) or of the UL34-35 (All (UL33, 36-38)) ORFs produced 212.9 ± 33.3 and 107 ± 5.2%, respec-

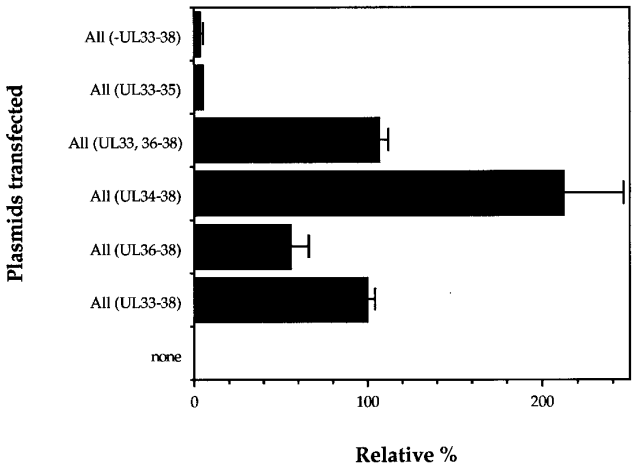

FIG. 2. Transactivation of the HCMV UL54 promoter construction by UL36-38 gene products. HFF cells were lipofected with pAI13 (UL54 promoter), All (TRS1, UL84, IE1, IE2, and UL112-113 expression vectors), and the genomic constructions encoding UL33-38 (pZP8), UL36-38 (pAI9, ΔUL33-35), UL34-38 (pAI10, ΔUL33), and UL33, 36-38 (pAI11, ΔUL34, 35). Control cultures were transfected with pAI13 and All only (All (−UL33-38)). Cells were harvested 96 h after lipofection and assayed for luciferase activity. Shown is a representative experiment with the averages of triplicate samples. The error bars indicate standard errors of the means.

tively, and, therefore, had no reductive effect on UL54 regulation. The simultaneous deletion of UL33, 34, 35 ORFs (All (UL36-38)) had a modest effect on UL54 regulation, reducing this value to 55.9 ± 10.2%. However, deletion of the UL36-38 ORFs (All (UL33-35)) abrogated regulation of the UL54 promoter reducing the relative value to 4.8 ± 0.7%. This value was similar to that (3.8 ± 14%) obtained in the absence of the complete UL33-38 construction (All (−UL33-38)). We conclude, therefore, that the UL36-38 region is largely responsible for the cooperative activation of HCMV early gene promoters by the products of the UL33-38 locus.

To determine which of the UL36-38 products was responsible for the transactivation, we tested the pooled UL36, UL37x1/38, UL37, and UL38 cDNAs in combination with All (Fig. 3). For these experiments, we show transactivation of the srt and UL57 promoter constructions. However, similar results were obtained with the UL44, UL54, and UL112-113 promoter constructions (unpublished observations). Combination of All with the pooled UL36-38 cDNAs (All (UL36-38 cDNAs)) provided 100 ± 37.2 and 100 ± 30.4% for the srt and UL57 promoter constructions, respectively. Removal of only the UL37x1/UL38 cDNA (All (−UL37x1/38)) abrogated activity as the relative conversion was reduced to 5.0 ± 15% (srt promoter) and 10.2 ± 4.8% (UL57 promoter). Similarly, cells transfected with All and an acidic domain deletion mutant of pUL37x1 (All (UL37x1 Δaa 53–140) had reduced activity for both the srt (10.5 ± 7.0%) and UL57 (10.1 ± 6.4%) promoter constructions. In contrast, deletion of the UL38 ORF (All (UL37x1), 86.27 ± 83.1, 93.8 ± 33.6%) or its truncation (All (UL37x1/UL38 stop), 88.0 ± 11.9, 69.4 ± 6.0%) did not reduce activity of the encoded proteins. Thus, pUL37x1 activated the expression of the HCMV early gene promoter constructions. In particular, these results implicate the pUL37x1 acidic domain as being critical for the regulation of HCMV early gene promoters.

To determine whether any individual UL36-38 cDNA is sufficient to replace the genomic UL33-38 construction, we tested addition of each individual cDNA in the All mixture (Fig. 4A). Addition of the pUL37x1/38 cDNA (All (UL37x1/38)) had the most marked stimulatory effect, providing about 62.4 ± 10.2% of the activity of the total genomic control (All (UL33-38), 100 ± 11.1%), while the gpUL37 had a modest but notable and consistent effect (18 ± 3.8%) on the UL44 promoter construction. Addition of either pUL36 (All (UL36), 6.7 ± 0.5%) or pUL38 (All (UL38), 7.2 ± 2.9%) expression clones did not have a measurable stimulatory effect on UL44 gene expression. Thus, transactivation of the HCMV early UL44 gene promoter construction by UL33-38 results predominantly from the transactivation effect of pUL37x1 and, to a lesser degree, from that of gpUL37.

Transfection of the individual pUL37x1 or gpUL37 expression clones, in the absence of other HCMV regulatory proteins, provided a minimal transactivation of the HCMV UL44 promoter construction (Fig. 4B). pUL37x1 and gpUL37 increased the expression of the UL44 promoter construction 0.5 ± 0.1 and 0.3 ± 0.0%, respectively. These levels were about two- to threefold

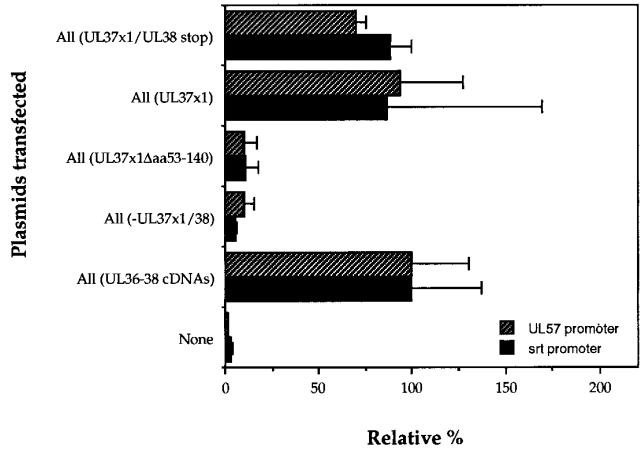

FIG. 3. Mutations in pUL37x1 eliminate transactivation, but mutations in pUL38 have minimal effect. HFF cells were lipofected with luciferase expression vectors pLHB135 (srt promoter) or pLHB2 (UL57 promoter) and All (TRS1, UL84, IE1 and 2, UL112-113) as well as the pooled UL36-38 cDNAs (All (UL36-38 cDNAs)) or with the pooled cDNAs but without the UL37x1/38 cDNA (All (−UL37x1/38)). Mutant constructions lacking the acidic domain (UL37x1Δaa 53–140), or the UL38 ORF (UL37x1), or with a truncation of the UL38 ORF (UL37x1/38 stop) were used in place of the wild-type UL37x1/38 cDNA. Control cultures were transfected with the respective luciferase expression vector alone (none). Shown is a representative experiment with the averages of triplicate samples. The error bars indicate standard errors of the means.

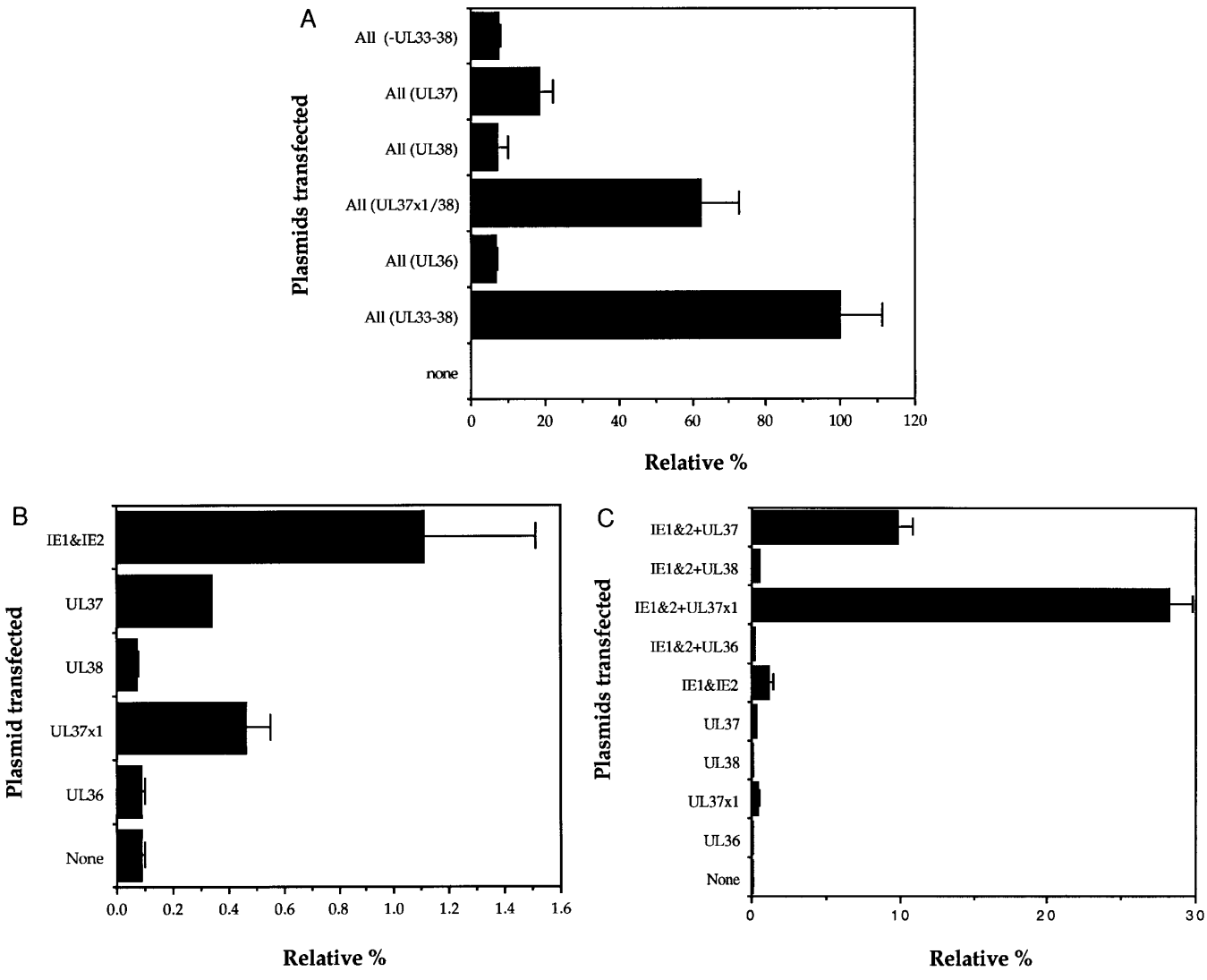

FIG. 4. pUL37x1 transactivates independently and cooperates synergistically with the major immediate early proteins. (A) HFF cells were lipofected with pAI1 (UL44 promoter construction), All (TRS1, pUL84, IE1, IE2, UL112-UL113), and pZP8 (All (UL33-38)), the indicated individual cDNAs, or without pZP8 (All (−UL33-38)). Control cultures were transfected with the pAI1 vector alone (none). HFF cells were lipofected with pAI1 (UL44 promoter) and (B) indicated individual expression vectors or with no additional DNA (none) or (C) with a combination of IE1 and IE2 and the individual indicated cDNAs. Shown is a representative experiment with the averages of triplicate samples. The error bars indicate standard errors of the means.

less than the combined effect of IE1 and IE2 (1.1 ± 0.4%) on the UL44 promoter construction. No regulation was observed following transfection with the UL36 (0.1 ± 0.01%) or UL38 (0.1 ± 0.01%) expression vectors when compared to addition of no expression vector (none, 0.1 ± 0.01%).

The degree of regulation was greatly increased by the combination of the pUL37x1 expression vector and the IE1 and IE2 expression vector (Fig. 4C). Synergistic activation of the UL44 gene promoter construction is observed between the MIE IE1 and IE2 proteins and pUL37x1 (28.2 ± 1.6%) and, to a lesser degree, with gpUL37 (9.8 ± 1.6%). While no synergism of IE1 and IE2 was observed with either pUL36 (0.2 ± 0.1%) or pUL38 (0.5 ± 0.0%). These effects are noted for a broad spectrum of HCMV early gene promoters, ranging from the candidate srt promoter to those of the UL44 and UL57 genes. Taken together with those above, these results suggest that regulation of HCMV early gene promoters requires multiple IE proteins from different loci, in particular IE1 and IE2 with pUL37x1 or gpUL37.

To test the requirement for the gpUL37 acidic domain, common to gpUL37 and pUL37x1 and found to be critical for pUL37x1 activity, we tested a gpUL37 acidic domain deletion mutant for transactivation of the HCMV UL44 gene promoter construction (Fig. 5). This mutant (gpUL37 Δaa 53–140) is known to traffic through the secretory apparatus similarly to wild-type gpUL37 and to retain most of the wild-type transactivating activity for the *hsp*70 promoter construction (Zhang *et al.,* 1996). Wild-type gpUL37, in combination with IE1 (UL37 + IE1),

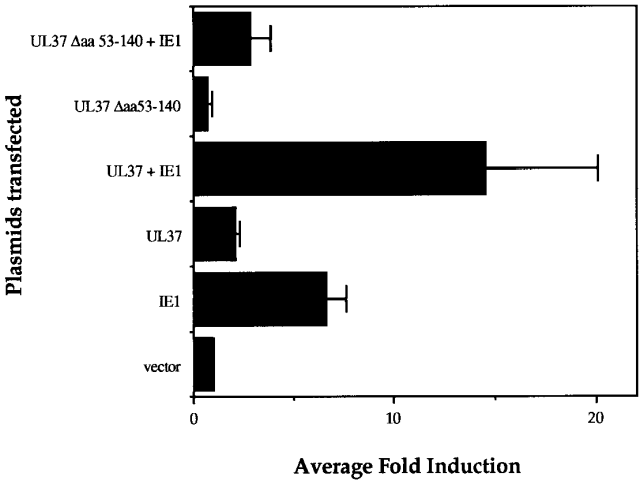

FIG. 5. The gpUL37 acidic domain is required for transactivation of the UL44 promoter. HFF cells were lipofected with pAI1 (UL44 promoter) and the indicated expression vectors. The cells were harvested 96 h posttransfection and assayed as described under Materials and Methods. Results show the means and standard errors of triplicate samples obtained in four independent experiments.

stimulated the UL44 promoter construction about 14.6 (±5.5)-fold above the levels observed with vector control (1.0 ± 0.0-fold). This value was increased above either IE1 (6.6 ± 1.0-fold) or gpUL37 (UL37, 2.1 ± 0.2-fold) alone. Deletion of the acidic domain abrogated activity of the gpUL37 protein alone (UL37 Δaa 53–140, 0.7 ± 0.2-fold) or in combination with IE1 (UL37 Δaa 53–140 + IE1, 2.8 ± 1.0-fold). Thus, similar to the findings with pUL37x1, the acidic domain of gpUL37 appears to play a critical role in transactivation of HCMV early gene promoters.

To determine the requirement for gpUL37 unique sequences in UL44 early gene promoter transactivation, a truncation mutant lacking all of the unique sequences of gpUL37 downstream of aa 199 (gpUL37 aa 1–199) or another lacking the gpUL37 carboxy-terminal cytosolic tail (gpUL37 aa 1–461) were assayed for their ability to transactivate the UL44 gene promoter construction in the presence or the absence of IE1 (Fig. 6). gpUL37 aa 1–199 alone (UL37 aa 1–199, 2.2 ± 0.4-fold) or in combination with IE1 (UL37 aa 1–199 + IE1, 17.0 ± 4.4-fold) transactivated the HCMV UL44 promoter construction to levels comparable to those obtained with wild-type gpUL37 alone (UL37, 2.1 ± 0.2-fold) or with wild-type gpUL37 and IE1 (UL37 + IE1, 14.6 ± 5.5-fold). Use of a gpUL37 mutant lacking the cytosolic residues produced similar results. gpUL37 aa 1–461 alone (UL37 aa 1–461, 4.1 ± 1.9-fold) or in combination with IE1 (UL37 aa 1–461 ± IE1, 17.0 ± 4.4-fold) transactivated the UL44 gene promoter construction to levels similar to those obtained with the wild-type protein. These results indicate that the unique domains (N-glycosylation, basic, transmembrane, and cytoplasmic domains) lacking in these mutants are not necessary for transactivation of the HCMV early gene promoter constructions.

DISCUSSION

In these studies, we have defined the members of the UL36-38 products which are capable of cooperative regulatory interactions in the transactivation of HCMV early gene promoters. Our results suggest that the requirement for UL36-38 proteins for HCMV DNA replication may result from the ability of pUL37x1 and, to a lesser extent, of gpUL37 to transactivate HCMV DNA replication gene promoters. The acidic domain of either of these proteins appears to play a critical role for these regulatory interactions.

HCMV IE loci, including the UL36-38 locus, were found to be required for HCMV *ori*Lyt DNA replication when the DNA replication genes were expressed under the control of their own promoters (Pari and Anders, 1993; Pari *et al.,* 1993). Sarisky and Hayward (1996) identified a strict requirement for both UL84 and UL36-38 products in an *ori*Lyt-directed omission assay. Nevertheless, the requirement for UL36-38 products, but not UL84, was eliminated by supplementing the transient assays with ppUL69 in Vero cells. ppUL69 is a virion protein which localizes to nuclear compartments and DNA replication compartments and is presumed to transactivate gene expression broadly by interacting with a chromatin factor (Winkler *et al.,* 1994; Sarisky and Hayward, 1996; Winkler and Stamminger, 1997). Nevertheless, it was not possible to replace UL36-38 products in the *ori*Lyt-directed omission assay in HFF cells, suggesting, in this latter case,

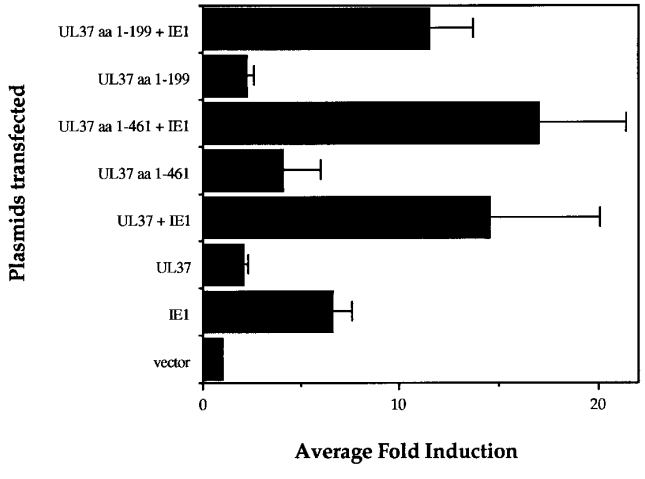

FIG. 6. The unique domain of gpUL37 are dispensable for UL44 promoter transactivation. HFF cells were lipofected with pAI1 (UL44 promoter) and the indicated expression vectors. The cells were harvested 96 h posttransfection and assayed as described under Materials and Methods. Results show the means and standard errors of duplicate samples obtained in four independent experiments.

incomplete complementation of UL36-38 function by ppUL69.

The requirement for UL36-38 products during *ori*Lyt DNA replication appears to result, at least in part, from the regulatory activities of the pUL37x1 and to a lesser degree of gpUL37. Based upon their regulatory activities, it is generally surmised that IE proteins alter the expression of cellular and viral genes which are required for the progression of the viral lifecycle. These studies demonstrate that pUL37x1 and gpUL37 can regulate HCMV early DNA replication gene promoter constructions, while the other UL36-38 IE protein, pUL36, did not. The requirement for such transactivators in DNA replication could be explained by the need to efficiently express the HCMV early promoters during HCMV DNA replication. The observed dependence upon cooperative regulator interactions of multiple loci for the efficient expression of cellular and viral replication proteins is consistent with our previous results (Iskenderian *et al.,* 1996).

Although IE1 served as co-transactivator in transactivation of both UL44 promoter and *hsp*70 promoter constructions (Zhang *et al.,* 1996), the phenotypes of the acidic domain and truncation gpUL37 mutants obtained with the HCMV early promoter constructions in HFF cells are in marked contrast to those obtained with the human *hsp*70 promoter construction (unpublished results). The gpUL37 aa 1–199 truncation mutant in combination with IE1 was inactive in *hsp*70 promoter transactivation (unpublished results). This was in spite of the finding that the gpUL37 aa 1–199 mutant retains almost wild-type activity with the HCMV UL44 gene promoter construction. These results suggest that, in contrast to HCMV early promoters, transactivation of the *hsp*70 promoter construction in HFF cells does not require the gpUL37 acidic domain (Zhang *et al.,* 1996) but does require unique gpUL37 sequences.

The pUL37x1 and gpUL37 proteins may act in a transcriptional or posttranscriptional capacity to activate expression of core genes. At the level of transcription, they may play a direct or an indirect role during transcription. We favor an indirect role, as the requirement for UL36-38 proteins can be overcome by the addition of ppUL69, in Vero cells, which is thought to transactivate gene expression by interacting with a chromatin factor (Sarisky and Hayward, 1996; Winkler and Stamminger, 1997).

Our previous studies establish that gpUL37 is synthesized as a type I integral membrane protein and is trafficking through the endoplasmic reticulum and the Golgi apparatus (Al-Barazi and Colberg-Poley, 1996). Thus, the acidic domain of gpUL37 is membrane-bound following synthesis of the primary translation product. However, the gpUL37 acidic domain could be released by proteolytic cleavage and translocated to nucleus. Processing by proteolytic cleavage of a membrane-bound regulatory protein and subsequent translocation to the nucleus is known to occur with sterol regulatory element binding proteins (Sakai *et al.,* 1996). There is a potential protease site on gpUL37 starting at aa 416, prior to the TM domain. However, our previous studies indicate that a large portion of gpUL37 (aa 201–420) is intact and not detectable in the cytosol by immunoprecipitation using polyvalent antibodies against the N-glycosylation domain (Al-Barazi and Colberg-Poley, 1996). Nevertheless, a small acidic subdomain, lacking those epitopes, would not have been detected by immunoprecipitation. Colocalization studies using confocal microscopy indicate that the carboxy terminus of gpUL37 does not detectably colocalize with the predominantly nuclear IE1 protein (Zhang *et al.,* 1996).

Although the pUL37x1 is virtually contained within gpUL37, pUL37x1 is likely to have a different processing and trafficking pattern than that of gpUL37. The N-glycosylation, transmembrane, and cytosolic domains in gpUL37 are not present in pUL37x1. Localization studies of pUL37x1 in HCMV-infected cells are confounded by the nearly complete overlap of its ORF with that of the amino-terminal 162 aa of gpUL37. However, it is likely that pUL37x1 can enter the nucleus because of its small size and the absence of anchor sequences. We note that there are several clusters of basic residues which might serve as nuclear localization signals.

Consistent with both proteins having an effect on HCMV early gene promoter constructions, albeit to a lesser degree with gpUL37, the acidic domain common to both of these proteins plays a key role in this regulation. Similarly, the acidic domains of IE2 are required for its function (Pizzorno *et al.,* 1991).

The pUL37x1/gpUL37 acidic domain may serve to recruit transcriptional machinery. Acidic activator proteins stimulate transcription by RNA polymerase II by a mechanism that is conserved among eukaryotic organisms (Tjian and Maniatis, 1994). Acidic activation domains can interact *in vitro* directly with some components of the general transcription machinery. These include the TATA-binding protein, TBP (Ingles *et al.,* 1991; Stringer *et al.,* 1990), TBP-associated factors (TAFs), which are components of the TFIID complex (Goodrich *et al.,* 1993; Hoey *et al.,* 1993), TFIIA (Ozer *et al.,* 1994), TFIIB (Lin *et al.,* 1991), TFIIF (Joliot *et al.,* 1995), and TFIIH (Xiao *et al.,* 1994). The acidic activation domain of Epstein–Barr virus nuclear antigen 2 associates with p100, a cellular protein that directly interacts with TFIIE (Tong *et al.,* 1995). In stepwise assembly reaction, acidic activation domains can stimulate formation of a TFIID–TFIIA–TATA element complex, recruitment of TFIIB, and the recruit-

ment of later acting components (StargeII and StruhI, 1996).

From their studies, Sarisky and Hayward (1996) suggest that UL36-38 proteins stimulate posttranscriptional events; however, our results showing the quantitatively differential transactivation of HCMV early promoter–luciferase constructions, which differ only in their promoter sequences, by gpUL37 argue that the transactivation is at the level of transcription. Consistent with these findings, a NF-kB-tk promoter–luciferase construction containing the luciferase gene, the SV40 intron, and polyadenylation signals, as used in the pGL2-basic vector used for these HCMV early promoter–indicator plasmids, is unresponsive to gpUL37 transactivation (unpublished observations). These results are consistent with gpUL37 transactivating the transcription of the HCMV early gene promoter constructions.

MATERIALS AND METHODS

Plasmid constructions

Figure 1B shows the wild-type gpUL37 or pUL37x1 motifs retained by the mutant proteins used for this study. p521 encodes gpUL37 Δaa 53–140 and has been previously described (Zhang *et al.,* 1996). p567 encodes gpUL37 aa 1–199. To generate p567, the UL37 cDNA from p414 (Colberg-Poley *et al.,* 1992) was cloned as an *Eco*RI fragment into pBSII(SK$^+$) (Stratagene) generating p523. The UL37 ORF was then mutated by the insertion of a stop linker (5′-CTA GGC CTT AGC GGC CGC TAG-3′) between the *Sna*BI sites [nucleotides (nt) 50,777–50,395] generating p528. The mutation was verified by DNA sequencing using the US Biochemical Sequenase kit. The mutant UL37 cDNA from p528 was subcloned into the p394 expression vector containing the HCMV MIE promoter (nt 173,678–174,402) and an SV40 polyadenylation site (nt 2533 to 2770) resulting in p567. p612 encodes gpUL37 aa 1–461. To generate p612, p414 DNA was mutated by the insertion of the stop linker above into the unique *Nru*I site (nt 49,991) in the UL37 ORF. The construction was verified by nucleotide sequencing.

Plasmid AI9 was made by treating pZP8 (Pari and Anders, 1993) with *Xho*I and *Bgl*II, blunting the ends with Klenow, and religating to delete the sequence between nt 42,045 and 47,366, containing the UL33, UL34, and most of the UL35 ORFs. Plasmid AI10 was made by treating pZP8 with *Xho*I plus *Nsi*I under conditions in which *Xho*I cleavage was complete but *Nsi*I cleavage was partial, gel purifying the fragment singly cut with *Nsi*I at nt 43,703, blunting with Klenow treatment, and self-ligating, removing most of the UL33 ORF. Plasmid AI11 was made by treating ZP8 with *Bst*1107I and *Xmn*I under conditions in which *Bst*1107I digestion was complete but *Xmn*I digestion was partial, gel purifying the plasmid singly cut with *Xmn*I at nt 45,448, blunting the ends with Klenow, and self-ligating, deleting portions of the UL34 and UL35 ORFs. Plasmid 327Δ*Age*I was made by treating p327 (Tenney and Colberg-Poley, 1991a) with *Age*I and self-ligating, removing sequences encoding a 88-amino-acid segment, including the pUL37x1 acidic region. Plasmid 327Δ*Mlu*I/*Nru*I was made by treating p327 with *Mlu*I plus *Nru*I, blunting the ends, and self-ligating, deleting the segment extending from nt 51,428 to 51,788 within the UL38 ORF. Plasmid 327*Mlu*I/*Pac*I was made by linearizing p327 with *Mlu*I, blunting, and ligating into the blunted site a phosphorylated oligonucleotide (5′-CCT ACT TAA TTA ATG ACT GAC TGA C-3′) containing a *Pac*I site and stop codons in all six reading frames. Plasmid LHB135 was made by excising the *Xho*I to *Not*I fragment extending from nt 92,636 to 92,891 from pSP50 (Anders *et al.,* 1992), blunting with Klenow, and ligating the gel-purified fragment into the *Sma*I site of pGL2-basic (Promega Corp.).

TABLE 1

Effector and Target Plasmids Used in These Experiments

Effector plasmid	ORFs	Reference
pSVH	IE1 and IE2	Stenberg *et al.* (1990)
pRR59	IE1	Colberg-Poley *et al.* (1991)
pZP8	UL33-38	Pari *et al.* (1993)
pZP24	UL112/113	Pari and Anders (1993)
pZP13	UL84	Pari *et al.* (1993)
pXEXX-6.1	TRS1	Thomas Jones (unpublished) Iskenderian *et al.* (1996)
pZP3	IRS1	Pari *et al.* (1993)
pAI9	ZP8ΔUL33-35	This work
pAI10	ZP8ΔUL33	This work
pAI11	ZP8ΔUL33-34	This work
pAI12	ZP8ΔUL36-38	Iskenderian *et al.* (1996)
p326	pUL36	Tenney and Colberg-Poley (1991a)
p327	pUL37x1/pUL38	Tenney and Colberg-Poley (1991a)
p327Δ*Age*I	pUL37x1Δ aa53-140	This work
p327Δ*Mlu*I/*Nru*I	pUL37x1/pUL38 stop	This work
p327/*Mlu*I/*Pac*I		This work
p406	pUL38	Colberg-Poley *et al.* (1991)
p414	gpUL37	Colberg-Poley *et al.* (1992)
p567	gpUL37 aa 1–199	This work
p521	gpUL37 Δaa 53–140	Zhang *et al.* (1996)
p612	gpUL37 aa 1–461	This work
Target plasmid	**Promoter–indicator**	**Reference**
pLHB135	srt promoter–luc	Huang *et al.* (1996)
pLHB2	UL57 promoter–luc	Iskenderian *et al.* (1996)
pAI1	UL44 promoter–luc	Iskenderian *et al.* (1996)
pAI3	UL70 promoter–luc	Iskenderian *et al.* (1996)
pAI5	UL102 promoter–luc	Iskenderian *et al.* (1996)
pAI7	UL105 promoter–luc	Iskenderian *et al.* (1996)
pAI13	UL54 promoter–luc	Iskenderian *et al.* (1996)
pAI15	UL112-113 promoter–luc	Iskenderian *et al.* (1996)

Transactivation experiments

Experiments were done essentially as described by Iskenderian *et al.,* (1996) as either addition or subtraction protocols, using the indicated target and effector plasmids listed in Table 1. Briefly, HFF cells were lipofected with a total of 1 μg of DNA using Lipofectamine (GIBCO-BRL, Iskenderian *et al.,* 1996). Cells were harvested in lysis buffer (Promega, E3971) and assayed for luciferase activity using Promega reagents (E1483). The production of photons was measured using either a beta scintillation counter with the coincidence counting circuit turned off or a beta scintillation counter with a Single Photon Monitor (Beckman Instruments, Colberg-Poley *et al.,* 1998).

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