Human cytomegalovirus IE86 protein interacts with promoter-bound TATA-binding protein via a specific region distinct from the autorepression domain

JOURNAL OF VIROLOGY, Dec. 1993, p. 7539–7546
0022-538X/93/127539-08$02.00/0

Vol. 67, No. 12

Human Cytomegalovirus IE86 Protein Interacts with Promoter-Bound TATA-Binding Protein via a Specific Region Distinct from the Autorepression Domain†

RAY JUPP,[1] STEFAN HOFFMANN,[1] RICHARD M. STENBERG,[2] JAY A. NELSON,[1]* AND PETER GHAZAL[3]

Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon 97201[1]; Department of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk, Virginia 23501[2]; and Departments of Immunology and Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, California 92037[3]

Received 22 March 1993/Accepted 25 August 1993

The major immediate-early gene of human cytomegalovirus encodes several isoforms of an immediate-early protein which has distinct transcriptional regulatory properties. The IE86 isoform autorepresses the major immediate-early promoter by directly binding the *cis* repression signal element located between the TATA box and the mRNA cap site. In addition to this activity, IE86 stimulates other viral and cellular promoters. One mechanism by which eukaryotic regulatory proteins are thought to stimulate transcription is by contacting one or more general transcription factors. We show that the IE86 protein physically interacts with the DNA-binding subunit (TATA-binding protein) human transcription factor IID via the TATA-binding protein-contacting domain in the N terminus of IE86. In a mobility shift assay, IE86 was also observed to stabilize the binding of TATA-binding protein to promoter DNA. The domains within IE86 responsible for mediating transactivation and repression functioned independently. These experiments thus demonstrate the elegant ability of human cytomegalovirus to join different protein domains to produce distinct multifunctional proteins.

The immediate-early (IE) genes of human cytomegalovirus (HCMV) encode transcriptional regulatory factors which, together with host cell proteins, temporally regulate subsequent viral gene expression (4, 10, 40, 41, 43–45, 47). We are interested in understanding the role of viral and cellular proteins involved in coordinating RNA polymerase II activity associated with HCMV gene regulation.

Three predominant IE protein isoforms originate from an abundant region of IE expression which is controlled by the major IE promoter (MIEP) (5, 42, 44, 45). These isoforms are generally distinguished by their apparent molecular masses of 72 (IE72), 86 (IE86), and 55 (IE55) kDa. Since these IE protein isoforms are all derived from a single precursor mRNA by differential splicing and polyadenylation site usage, the virus can join various domains to create proteins with similar and distinct functions. Recently, IE86 was shown bind the *cis* repression signal (crs) element located between the TATA box and the mRNA cap site of the MIEP (21, 24). The IE86 protein possesses a domain between amino acid residues 365 and 519 which contains a putative zinc finger and a leucine-rich region (Fig. 1B) that was shown to be critical for this interaction (21, 32). The crs element is responsible for conferring negative repression on the MIEP by IE86 (7, 16, 30, 36, 38).

Although IE86 and the TATA-binding protein (TBP) were observed to bind DNA simultaneously, binding of one protein impaired the binding of the other (21). Although IE86 may effect the binding of transcription factor IID (TFIID), the multisubunit complex containing TBP, to the crs element, the main block in transcription is likely at the level of TFIIB recruitment (21). The IE55 protein, however, lacks the domain in IE86 between amino acid residues 365 and 519 and consequently is unable to bind the crs element and repress MIEP activity (1, 21). Therefore, IE55, like IE72, which also does not possess this domain, is a transactivator rather than a repressor of MIEP activity (1, 8, 16, 39). By contrast, IE86, IE55, and IE72 are all capable of transactivating heterologous promoters (1, 10, 14, 17, 20, 43, 48, 49). Together, the IE86 and IE72 proteins synergistically transactivate different kinetic classes of HCMV promoters as well as other viral and cellular promoters (9, 14, 17, 43, 47–49). Thus, the relative levels of these proteins may be critical for determining the outcome of viral infection.

The mechanisms by which the IE proteins interact with host proteins to coordinate viral transcription are not clearly understood. Evidence from studies on other viral IE proteins suggest that the HCMV IE proteins may interact with basal or sequence-specific transcription factors or by direct binding to DNA. For example, the large adenovirus E1A protein binds both the sequence-specific transcription factor ATF-2 and TBP, from which it was suggested that E1A stimulated transcription through TBP after recruitment to the promoter by ATF-2 (19, 26, 31). The Zta (ZEBRA, EB1, or BZLF1) protein from Epstein-Barr virus binds a DNA motif upstream of the target promoter and appears to stimulate transcription by stabilizing the binding of TBP to DNA (27).

Recently, IE86 was shown to transactivate an early HCMV promoter in nuclear extracts prepared from U373-MG, HeLa, and *Drosophila* embryo cells (23). These *Drosophila* embryo nuclear extracts contained relatively small amounts of nonspecific DNA-binding proteins. Addition of histone H1 to these extracts resulted in greater transactivation of this early promoter by IE86, suggesting that activation by IE86 involves antirepression (23). In addition, IE86 has also been shown to interact directly with TBP by coimmunoprecipitation and Western blot (immunoblot) analysis in the absence of DNA (15). However, the conformation adopted by TBP in these studies is likely different from that adopted when TBP is bound

* Corresponding author.

† Publication no. 7894-IMM from the Departments of Immunology and Neuropharmacology, Scripps Research Institute.

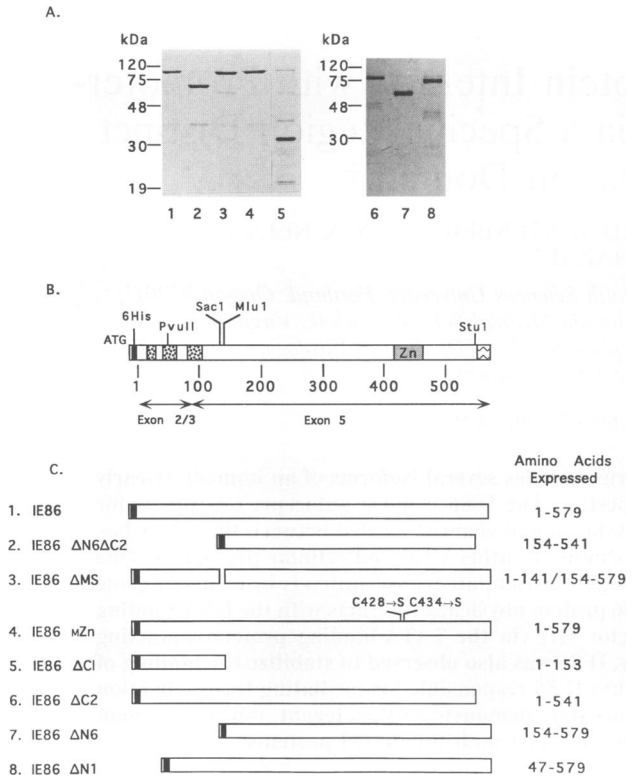

FIG. 1. Wild-type and mutant forms of IE86 and human TBP. (A) Coomassie-stained gel showing the wild-type and mutant forms of IE86 after nickel chelate chromatography and dialysis of pooled fractions containing purified protein. Lane 1, wild-type IE86; lanes 2 to 8, mutant forms IE86ΔN6ΔC2, IE86ΔMS, IE86mZn, IE86ΔC1, IE86ΔC2, IE86ΔN6, and IE86ΔN1, respectively. (B) Schematic representation of the HCMV IE86 protein. The three amphipathic helices are indicated by the shaded regions between amino acids ~30 and 105. The zinc finger (Zn) acidic region, represented by the shaded region between amino acids 551 and 579, the six-histidine tag (6His), and the restriction sites used in the construction of the expression vectors are also shown. (C) Schematic representations of wild-type and mutant IE86 proteins. The amino acids expressed, together with the six-histidine tag and initial ATG codon, are indicated.

to DNA (22, 34). In addition, an IE86-glutathione-*S*-transferase fusion protein was used to coprecipitate TBP from crude nuclear extracts of human embryonic lung cells (12). These studies indicate the significance of this interaction under more physiological conditions. Therefore, there are two possible mechanisms which IE86 may stimulate transcription. Analysis of the domains responsible for these interactions is critical for the elucidation of IE86 function.

We have examined the ability of a panel of purified mutant IE86 proteins to interact with purified human TBP. We found that a specific domain in the N terminus of IE86 was responsible for binding TBP. Moreover, the N-terminal domain bound TBP in the absence of the domain responsible for repression, demonstrating their independence and the versatile nature of a virus that is able to join various protein domains.

MATERIALS AND METHODS

Plasmid constructions. The expression plasmid p86,6His ΔN6 was constructed by digesting p86,6His (21) with *Mlu*I, filling in the ends with Klenow polymerase, ligating 8-bp *Bam*HI linkers (New England Biolabs, Beverly, Mass.), and digesting with *Bam*HI. The fragment released was subcloned back into the *Bam*HI-digested p86,6His vector, and the sense orientation was selected. Plasmid p86,6HisΔN6ΔC2 was generated by digesting p86,6HisΔN6 with *Stu*I and *Hin*dIII, filling in the ends with Klenow polymerase, and ligating them back together. The *Hin*dIII site and sequence immediately downstream encodes stop codons in all three reading frames. Plasmid p86,6HisΔMS was constructed by digesting p86,6His with *Sac*I and *Mlu*I, treating the ends with mung bean exonuclease, and ligating them back together. The remaining plasmids expressing mutant forms of IE86 were described previously (21). The truncated form of TBP (TBPΔN1) was generated by digesting phTBP,6His (21) with *Pst*I and *Pml*I. The annealed oligonucleotides 5′-GTGGCAGTGGCAGCT GCA-3′ and 5′-GCTGCCACTGCCAC-3′, which had *Pml*I- and *Pst*I-compatible ends, respectively, were subsequently inserted to juxtapose Val-97 (GCA) of full-length TBP with the six-histidine tag. All cloning steps were confirmed by direct sequencing of the plasmid DNA.

Purification of recombinant proteins. Purification of the IE86 and TBP recombinant proteins has been described previously (21). Briefly, *Escherichia coli* strains harboring each of the expression plasmids were grown to an optical density at 550 nm of 0.7 to 0.8 before induction with 200 μg of isopropyl-β-D-thiogalactopyranoside (IPTG) per ml. Cells were harvested after 90 to 120 min and lysed in buffer containing 50 mM sodium phosphate (pH 7.8), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1% Tween 20, 1 M NaCl, and 1 mg of lysozyme per ml for 20 min prior to sonication. After centrifugation, the cleared lysate was subjected to Ni^{2+} chelate chromatography (Qiagen, Chatsworth, Calif.) over a column equilibrated in buffer containing 50 mM sodium phosphate (pH 7.8), 500 mM NaCl, and 10% glycerol. After the column was washed, fractions were eluted in the same buffer containing 500 mM imidazol at pH 6.0. Fractions containing each of the proteins were pooled and dialyzed against either 50 mM sodium phosphate (pH 7.6)–250 mM NaCl–1 mM β-mercaptoethanol–30% glycerol or 20 mM HEPES (hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 8.0)–0.5 mM EDTA–2 mM $MgCl_2$–1 mM β-mercaptoethanol–50 mM KCl–30% glycerol for the HCMV IE proteins and human TBP, respectively. Purified proteins were analyzed on 12% polyacrylamide–sodium dodecyl sulfate (SDS) gels and stained with Coomassie blue; concentrations were then determined by the Bradford method.

Mobility shift assay. The mobility shift assay was performed essentially as described previously (13) except that the assay buffer contained 25 mM Tris-HCl (pH 8.0), 0.5 mM dithiothreitol, 0.5 mM EDTA, 6.25 mM $MgCl_2$, 0.2 μg of poly(dG:dC), 10% glycerol, and cold competitor oligonucleotides as indicated, in a total volume of 25 μl. Probe PPM1 consisted of oligonucleotides 5′-AGCTTCATTGGGGAGGTCTATATA AGCAGAGCT-3′ and 5′-AGCTCTGCTTATATAGACCTC CCCAATGAAGCT-3′, which contained the HCMV MIEP TATA box but only part of the crs element. The wild-type and mutant forms of IE86 were incubated for 2 min in assay buffer before the addition of 0.5 pmol of probe which had been labeled with Sequenase (USB) and [α-^{32}P]dATP. The incubation was continued for 10 min before the mix was loaded onto a 4% polyacrylamide gel. Assay mixes which contained antibody were incubated for 2 min after the addition of probe before the addition of antibody. Incubation of these assay mixes was then continued for a further 10 min. Monoclonal antibodies to IE86 which recognized epitopes between amino acid residues 1 and 47 and 47 and 153 were raised in mice with

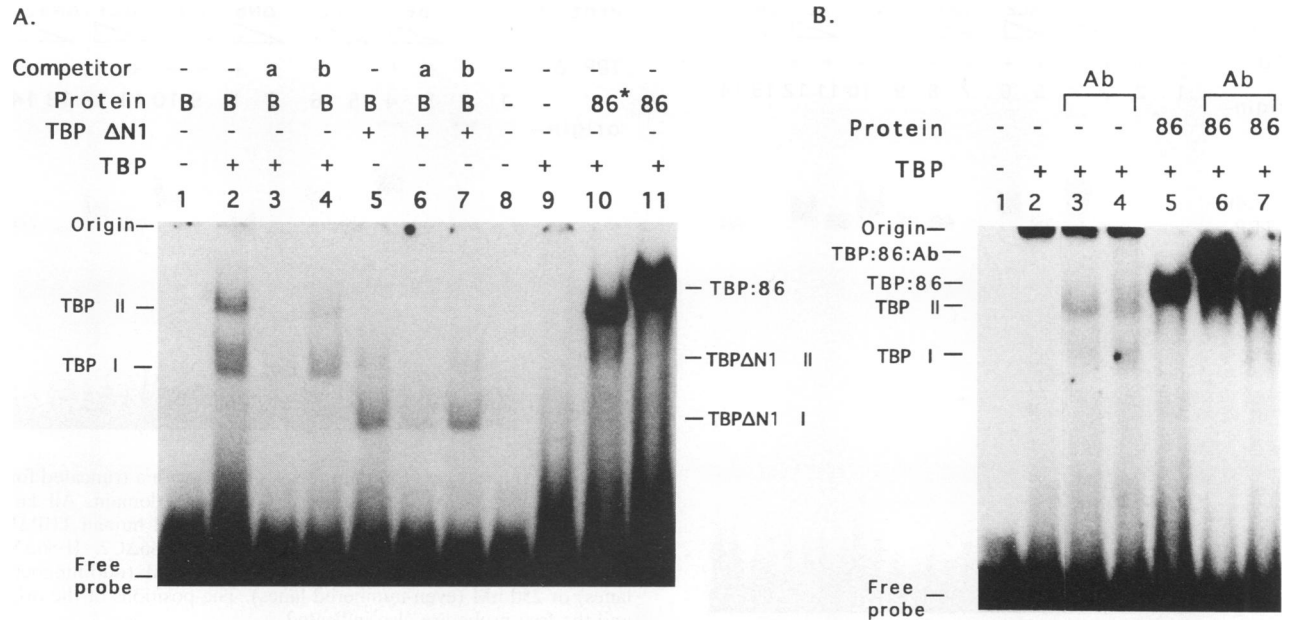

FIG. 2. Mobility shift assay demonstrating the interaction of IE86 with TBP on promoter DNA. All lanes in both panels contained 0.5 pmol of the PPM1 probe. (A) Lanes 2 to 4 and 9 to 11 also contained 50 nM human TBP, and lanes 5 to 7 contained an N-terminal truncation of TBP (TBPΔN1), as indicated by a +. BSA (500 μM) was included in lanes 1 to 7 to help stabilize the binding of TBP. The presence of BSA was not observed to effect the migration pattern of TBP. In addition, lanes 3 and 6 and lanes 4 and 7 contained a 15-fold excess of unlabeled PPM1 and 5′-TTGTGTAGGCCACGTGACCGGGT-3′ (encompassing a USF binding site), respectively. Lanes 10 and 11 also included 125 nM IE86 (86*) and 250 nM IE86 (86), respectively. The monomeric (complex I) and dimeric (complex II) forms of promoter-bound wild-type TBP and mutant TBPΔN1 are indicated. (B) Lanes 2 to 5 included 50 nM human TBP, and lanes 5 to 7 also included 250 nM IE86. Monoclonal antibodies to IE86 (epitope between residues 1 and 47), (lanes 3 and 6) and to gp120 of human immunodeficiency virus (lanes 4 and 7) were also present in the assay at a concentration of 40 μg/ml. The positions of the TBP-IE86 (TBP:86) and the TBP-IE86-antibody (TBP:86:Ab) nucleoprotein complexes are also indicated, together with the origin of the gel and the free probe.

purified IE86 as the antigen (20a). The gel and running buffer contained 6.7 mM Tris-HCl (pH 7.3), 3.3 mM sodium acetate, and 1 mM EDTA. After electrophoresis, the gel was dried and autoradiographed.

RESULTS

IE86 physically interacts with and promotes binding of TBP to DNA. Recombinant IE86 and TBP were expressed in and purified from *E. coli* as described in Materials and Methods. Purification was facilitated by the introduction of a short linker sequence encoding six histidine residues at the 5′ end of each cDNA. The six-histidine tag allowed the protein to be purified by nickel chelate chromatography. After purification, both IE86 and TBP were observed to be approximately 90% homogeneous (Fig. 1A, lane 1, and data not shown, respectively). The interaction of TBP with a fragment of the MIEP from −43 to −19 (PPM1) was examined in a mobility shift assay (Fig. 2A, lane 2). The inclusion of bovine serum albumin (BSA; 500 μM) in the reaction buffer helped to stabilize the binding of TBP to DNA (Fig. 2A, compare lanes 2 and 8), as demonstrated previously (27). Two complexes containing TBP, which correspond to the monomeric and dimeric forms of TBP (TBP I and II, respectively) bound to DNA, as described previously (22). Inclusion of BSA did not affect the migration pattern of the monomeric or dimeric forms of TBP.

Competition experiments demonstrated the specificity of these complexes (Fig. 2A, lanes 3 and 4). Excess unlabeled PPM1 probe competed for the binding of labeled probe to TBP (Fig. 2A, lane 3), whereas excess unlabeled probe containing a USF binding site did not (Fig. 2A, lane 4). Similar experiments were performed with a deletion mutant of TBP (Fig. 2A, lanes 5 to 7). This protein, TBPΔN1, was composed of amino acids residues 97 to 357 of wild-type TBP. This protein also yielded two complexes (TBPΔN1 I and II) which migrated with a faster mobility than wild-type TBP because of the loss of 96 N-terminal amino acids (Fig. 2A, compare lanes 5 and 2). The competition experiments shown in Fig. 2A, lanes 6 and 7, demonstrate the specificity of the TBPΔN1 nucleoprotein complexes. Unlabeled excess PPM1 competed for binding, whereas the probe containing the USF binding site did not compete for binding to TBPΔN1. Identical results were obtained with TBP lacking the six-histidine tag (data not shown).

The PPM1 probe contained a small amount of the sequence between −24 and −19 that was previously shown to be protected by IE86 during DNase I digestion (21, 24). However, the IE86 protein was not observed to bind this probe directly (data not shown). On this short exposure, binding of TBP to DNA was not readily visible (Fig. 2A, lane 9) and had to be stabilized by the inclusion of BSA to be observed (Fig. 2A, lane 2). By contrast, a more dramatic stabilization of the TBP II nucleoprotein complex was observed in the presence of 125 nM IE86 (Fig. 2A, lane 10). Since little of the TBP I nucleoprotein complex remained, the effect of IE86 was a preferential stabilization of the TBP II complex, a phenomenon not observed with BSA (Fig. 2A, compare lanes 2 and 10). Addition of 250 nM IE86, however, was required to form a stable IE86-TBP nucleoprotein complex of slower mobility (Fig. 2A, lane 11). Titration of IE86 into the reaction mix at concentrations between 125 and 250 nM resulted in a gradual

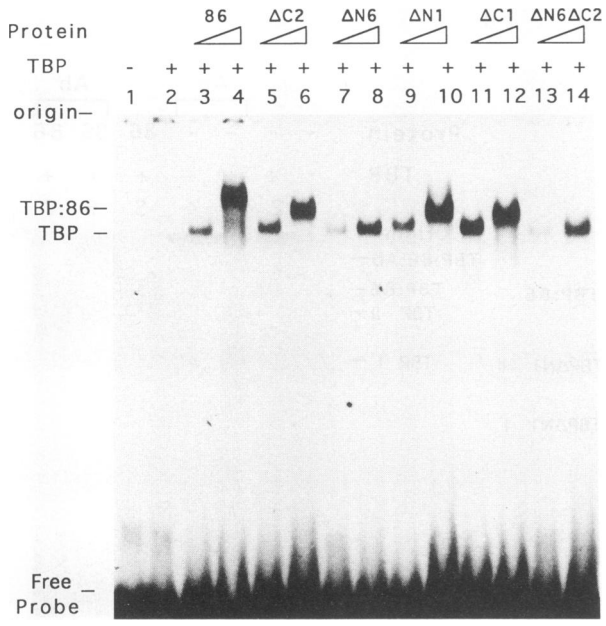

FIG. 3. Interaction of mutant forms of IE86 with TBP. All lanes contained 0.5 pmol of the PPM1 probe and 50 nM human TBP except lane 1, as indicated. In addition, 125 nM (odd-numbered lanes) and 250 nM (even-numbered lanes) of IE86, IE86ΔC2, IE86ΔN6, IE86ΔN1, IE86ΔC1, and IE86ΔN6ΔC2 were used. The positions of the nucleoprotein complexes corresponding to TBP and TBP-IE86 (TBP:86) are indicated. The mutant IE86-TBP nucleoprotein complexes were observed to migrate between the TBP and wild-type IE86-TBP complexes because of their smaller size. The positions of the origin and the free probe are also indicated.

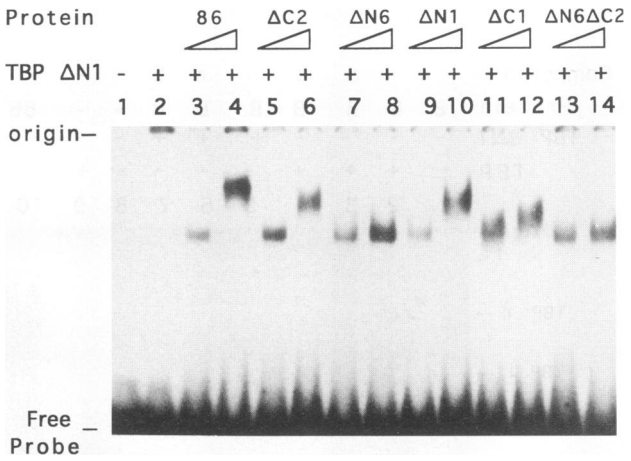

FIG. 4. Interaction of mutant forms of IE86 with a truncated form of TBP consisting of the conserved C-terminal domain. All lanes contained 0.5 pmol of the PPM1 probe and 50 nM human TBPΔN1 (except lane 1), as well as indicated. IE86, IE86ΔC2, IE86ΔN6, IE86ΔN1, IE86ΔC1, and IE86ΔN6ΔC2 at 125 nM (odd-numbered lanes) or 250 nM (even-numbered lanes). The positions of the origin and the free probe are also indicated.

increase in the amount of supershifted versus stabilized TBP (data not shown).

To demonstrate that IE86 is a part of the IE86-TBP nucleoprotein complex, a monoclonal antibody that recognized an epitope between amino acid residues 1 and 47 of IE86 was included in the assay. This monoclonal antibody supershifted the reaction containing 250 nM IE86 and 50 nM TBP together with probe (Fig. 2B, compare lanes 5 and 6). By contrast, a supershift was not observed with a control antibody which recognized the gp120 envelope protein of human immunodeficiency virus (Fig. 2B, lane 7). Addition of either antibody to the assay in the absence of IE86 did not supershift the TBP nucleoprotein complex (Fig. 2B, lanes 3 and 4). In addition, a monoclonal antibody to human TBP was also capable of supershifting the TBP and TBP-IE86 nucleoprotein complexes (51). A minor stabilization of the two TBP nucleoprotein complexes was also observed in the presence of antibody (Fig. 2B, lanes 3 and 4). These complexes migrated below that of the stable IE86-TBP complex (Fig. 2B, lane 5). These data show that IE86 is present within the shifted IE86-TBP nucleoprotein complex and demonstrate that IE86 interacts with TBP bound to promoter DNA.

Interaction of mutant IE86 proteins with TBP and identification of the TCD. To delineate the domain in IE86 responsible for binding TBP, various mutant IE86 expression plasmids were constructed. After their expression in *E. coli*, all of the proteins were purified and resolved on polyacrylamide gels (Fig. 1A, lanes 2 to 8). These mutant forms of IE86 are also schematically represented in Fig. 1C. The IE86ΔC2 mutant, which lacks the extreme C-terminal acidic domain of IE86 (amino acids 542 to 579), showed the same ability to bind TBP as wild-type IE86, demonstrating that this region is not responsible for contacting TBP (Fig. 3, lanes 5 and 6). The IE86ΔC2-TBP complex migrated slightly faster than the wild-type IE86-TBP nucleoprotein complex because of the removal of the C-terminal acidic region (Fig. 3, compare lanes 6 and 4). Further truncation of amino acid residues from the C-terminal end yielded the IE86ΔC1 mutant (Fig. 1C, construct 5). This protein was also observed to stabilize and shift the TBP nucleoprotein complex (Fig. 3, lanes 11 and 12). The difference in the migration of the TBP and TBP-IE86ΔC1 nucleoprotein complexes, however, was marginal, because the IE86ΔC1 protein consisted only of amino acid residues 1 to 153 of wild-type IE86. Nevertheless, this indicates that the domain of IE86 responsible for interacting with promoter-bound TBP is present within the N terminus.

To confirm these results, two mutant forms of IE86 were produced with deletions in the N terminus (Fig. 1C, constructs 7 and 8). Removal of amino acids 1 to 46 of IE86 resulted in protein IE86ΔN1 (Fig. 1C, construct 7), which was still able to interact with TBP (Fig. 3, lanes 9 and 10). Further truncation of the N terminus of IE86 generated the mutant IE86ΔN6 (Fig. 1C, construct 8), which was unable to form a stable complex with TBP prebound to DNA (Fig. 3, lane 8). These data demonstrate that the TBP-contacting domain (TCD) is located between amino acid residues 47 and 153 of IE86. Since both N- and C-terminal mutant IE86 proteins stabilized TBP binding, more than one domain in IE86 may be responsible for this activity.

Comparison of TBPs from several different species revealed a variable N-terminal domain as well as a highly conserved C-terminal domain (34). The conserved C-terminal domain is composed of two direct repeat sequences separated by a basic region (18). All transcription-regulatory proteins examined, including IE86, have been shown to contact TBP via the conserved domain and not the variable N-terminal domain (3, 15, 25, 26). To obtain better resolution of the mutant IE86-TBP nucleoprotein complexes, the TBPΔN1 mutant, which lacks 96 N-terminal amino acids, was used in the mobility shift assay. The conserved domain of TBP was also shifted by

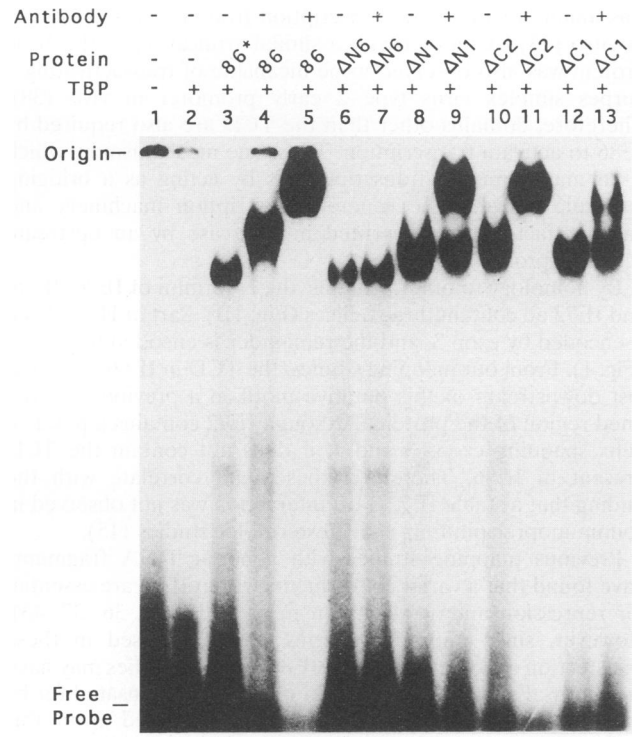

FIG. 5. Supershift of the mutant IE86-TBP nucleoprotein complexes by monoclonal antibody recognizing residues 47 to 153 of IE86. All lanes contained 0.5 pmol of PPM1 probe and 50 nM human TBP (except lane 1), as indicated. Lane 3 also contained 125 nM IE86 (86*). Lanes 4 to 13 all contained a 250 nM concentration of either wild-type or mutant forms of IE86, as indicated. Monoclonal antibody to residues 47 to 153 of IE86 was included in lanes 5, 7, 9, 11, and 13, as indicated, at a concentration of 200 μg/ml. The origin and position of the free probe are shown.

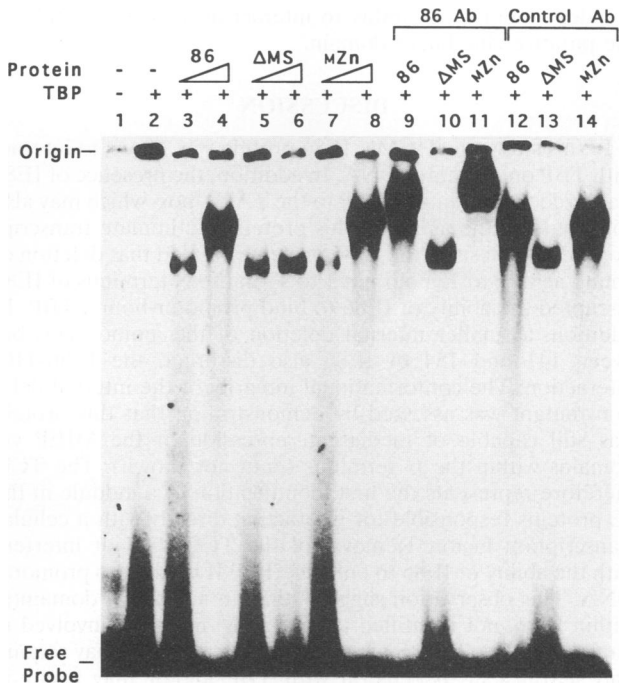

FIG. 6. Independence of the domains responsible for repression and activation. Mobility shift analysis of the interaction of IE86mZn and IE86ΔMS with TBP. All lanes contain the PPM1 probe (0.5 pmol) and 50 nM TBP where indicated (+). Lanes 3, 5, and 7 and 4, 6, and 8 contained 125 and 250 nM of IE86, IE86ΔMS, or IE86mZn (mZn), respectively. Probe was incubated with 250 nM IE86 (lanes 9 and 12), IE86ΔMS (lanes 10 and 13), and IE86mZn (lanes 11 and 14). In addition, monoclonal antibody (40 μg/ml) to the IE86 (residues 1 to 47) epitope or the control antibody to gp120 from human immunodeficiency virus was included in lanes 9 to 11 and 12 to 14, respectively. The origin and position of the free probe are indicated.

wild-type IE86 protein (Fig. 4, compare lanes 3 and 4). The mutant forms of IE86 which were able to form a stable complex with wild-type TBP were also able to interact with the truncated TBP (Fig. 4). Since mutant TBP migrated faster than wild-type protein, the stable TBPΔN1-IE86ΔC1 nucleoprotein complex was more clearly resolved from the complex containing just TBP and probe (Fig. 4, compare lanes 11 and 12). In addition, neither the IE86ΔN6 nor the IE86ΔN6ΔC2 protein was able to interact with the conserved domain of TBP (Fig. 4, lanes 8 and 14).

To conclusively demonstrate that the various mutant forms of IE86 stably interact with TBP, each of the mutant IE86-TBP nucleoprotein complexes was incubated with a monoclonal antibody which recognized an epitope in IE86 between amino acid residues 47 and 153. An identical supershift of the wild-type IE86-TBP nucleoprotein complex was obtained with this monoclonal antibody (Fig. 5, lane 5) as with the monoclonal antibody used in Fig. 2B, which recognized an epitope in IE86 between amino acid residues 1 and 47. In addition, the IE86ΔN1-TBP, IE86ΔC2-TBP, and IE86ΔC1-TBP complexes were also supershifted with the antibody against residues 47 to 153 (Fig. 5, lanes 9, 11, and 13, respectively). By contrast, this antibody did not supershift the control reaction containing IE86ΔN6, TBP, and probe (Fig. 5, lane 7). Also, a monoclonal antibody which recognized the mutant IE86ΔMS protein failed to develop a supershift in the presence of this protein, TBP, and probe, indicating that although IE86ΔMS was able to stabilize the TBP II complex, it was not present within the complex (Fig. 6A, lane 10; see below for details). Also, the IE86-stabilized TBP II complex had the same mobility as the TBP II complex in the absence of IE86 (Fig. 2A, compare lanes 2 and 10). Together, these data demonstrate that the TCD is present within the N terminus of IE86.

TBP-binding and repression properties of IE86 function independently. We have shown that a mutation in the putative zinc finger of IE86 which prevented IE86 from binding the crs element converted the protein from a repressor to an activator of MIEP activity in vivo (21). The zinc finger mutant of IE86, like wild-type IE86, was observed to bind to and shift the TBP nucleoprotein complex, consistent with previous findings (Fig. 6, compare lane 8 with lane 4). The IE86-TBP and IE86mZn-TBP complexes were both supershifted with the IE86 (residues 1 to 46) antibody (Fig. 6, lanes 9 and 11, respectively) but not with the control antibody (Fig. 6, lanes 12 and 14, respectively). To more closely map the domain in IE86 which contacts TBP, a mutant of IE86 containing a small internal deletion was expressed in and purified from *E. coli*. This mutant, IE86ΔMS, contained a deletion of 12 amino acids between amino acid residues 141 and 154 of IE86. This mutant was not observed to shift the TBP nucleoprotein complex (Fig. 6, lane 6). In addition, when either the IE86 (residues 1 to 47) or the control antibody was included in the assay with IE86ΔMS, supershift of the TBP nucleoprotein complex was not observed (Fig. 6, lane 13). Therefore, the ability of IE86 to bind to TBP on DNA

is independent of its ability to interact directly with DNA via the putative zinc finger domain.

DISCUSSION

In this study, the HCMV IE86 protein was shown to interact with TBP on promoter DNA. In addition, the presence of IE86 stabilized the binding of TBP to the TATA box, which may also contribute to the ability of this protein to stimulate transcription. Mutagenesis of the IE86 protein revealed that deletion of amino acids 1 to 153 but not 1 to 47 in the N terminus of IE86 disrupted the ability of IE86 to bind promoter-bound TBP. In addition, a smaller internal deletion of the amino acids between 141 and 154 of IE86 also disrupted the IE86-TBP interaction. The conformational integrity of the internal deletion mutant was assessed by demonstrating that this protein was still capable of mediating repression of the MIEP via domains within the C terminus (data not shown). The TCD therefore represents the first identification of a module in the IE proteins responsible for interacting directly with a cellular transcription factor. Removal of the TCD did not interfere with the ability of IE86 to enhance TBP II binding to promoter DNA. This observation suggests that an additional domain(s) within IE86, not identified in this study, might be involved in interacting with TBP. Such an interaction domain may require only a transient association with TBP and/or may occur in solution before TBP binds the TATA element. The TCD of IE86 was also shown to be critical for the ability of IE86 to activate transcription in vivo but was not involved in autorepression of the MIEP (data not shown). A mutant of IE86 carrying a mutation within the putative zinc finger module that failed to autorepress but instead enhanced transcription in vivo (21) was still capable of binding TBP. Therefore, this pleiotropic viral IE protein has two independent mechanisms by which it can recognize and influence a specific promoter. These two independent mechanisms involve distinct modules of the IE86 protein that recognize promoter DNA either by tethering IE86 to a DNA-bound protein or by mediating the direct interaction of IE86 with DNA.

Human TBP exists in vivo as a multiprotein complex (TFIID) in which the central DNA-binding subunit, TBP, is complexed with a variety of other polypeptides, known as TBP-associated factors (6, 11, 35). Recently, the interaction of IE86 with TFIID in vivo has been indicated by the ability of an IE86-glutathione-*S*-transferase fusion protein to coprecipitate TBP from crude nuclear extracts (12). The binding of IE86 to the C terminus of TBP fused to glutathione-*S*-transferase was also demonstrated by coimmunoprecipitation and Western blot analysis (15). In addition, an IE86-glutathione-*S*-transferase fusion protein was shown to transactivate an early HCMV promoter in vitro. After incubation of this promoter with histone H1, IE86-glutathione-*S*-transferase was observed to alleviate histone H1-induced repression of basal transcription (23). Whether IE86 counteracted the effect of histone H1 by a direct interaction or by enhancing the recruitment of RNA polymerase II via interaction with TBP without being impeded by histone H1-induced repression is unknown. The latter mechanism is similar to that suggested for the transcription factor USF (50). The TCD domain also encompasses a nuclear localization signal (37), suggesting that this domain may interact with additional factors and indicating the multifunctional role of this module. However, the extent to which the TCD and nuclear localization signal overlap is not known.

Interestingly, Klucher et al. also demonstrated that a mutant IE86-glutathione-*S*-transferase protein which lacked the C-terminal acidic domain and is thus comparable to IE86ΔC2 was unable to stimulate transcription from an early promoter in vitro (23). A mutant with a similar truncation of the IE86 protein was also observed to be incapable of transactivating a herpes simplex virus type 2 early promoter in vivo (38). Therefore, domains other than the TCD are also required by IE86 to activate transcription. Thus, one mechanism by which IE86 may stimulate transcription is by acting as a bridging molecule between the general transcription machinery and another factor(s), represented in this case by an upstream activator protein.

By homology to other proteins, the N termini of IE86, IE55, and IE72 all contain three helices (Fig. 1B). Part of H3 in IE86 is encoded by exon 3, and the remainder is encoded by exon 5 (Fig. 1). From our mapping studies, the TCD in IE86 is located just downstream of this putative motif in a previously undefined region of the protein. Although IE72 contains a putative helix spanning exons 3 and 4, it does not contain the TCD present in IE86. Therefore, these data correlate with the finding that a stable IE72-TBP interaction was not observed in coimmunoprecipitation and Western blot studies (15).

Previous mapping studies with genomic DNA fragments have found that a variety of elements within IE86 are essential for repression and/or activation in vivo (17, 33, 36, 37, 43). However, since multiple proteins were expressed in these transfection assays, mutation of IE86 in these studies may have indirectly affected the function of or been compensated for by one of the other regulatory proteins expressed from this region. Therefore, these studies can only serve as a reference to direct the mapping of IE86 interactions in a more defined system.

Other viral proteins also possess domains which have been shown to contact TBP. The transcriptional activator VP16 of herpes simplex virus type 1 was shown to interact with yeast TBP and TFIIB (29, 46). However, experiments designed to dissect the mechanism of VP16 action revealed that VP16 stabilized the binding of TFIIB to template DNA but had little effect on the binding of TBP (29). By contrast, the Zta protein from Epstein-Barr virus was shown to stabilize TBP binding to the TATA box via a direct physical interaction (27). In our studies, IE86 also stabilized the binding of TBP to template DNA, although the mechanism was independent of the TCD.

The adenovirus E1A and the IE86 proteins contain domains responsible for repression as well as activation (2, 26, 28). E1A (26) and the specific upstream DNA-binding protein ATF-2 (31) were shown to contact TBP and thus may function as bridging molecules, as we have proposed for IE86 (Fig. 6). Moreover, the activation and repression domains of E1A, like those in IE86, were shown to function independently, although the activation domain was dominant over the repression domain in E1A (28). These analyses show that viral regulatory proteins have adopted a number of common themes to coordinate their expression. Moreover, the mechanisms by which these proteins interact with those of the host frequently parallel host protein-host protein interactions. Thus, further investigation of the regulatory mechanisms of the HCMV IE proteins should add to our understanding of both viral and cellular gene regulation.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI-21640 and CA-50151 (J.A.N.) and AI-30627 (P.G.), and a Faculty Research Award from the American Cancer Society (J.A.N.).

We thank Diane Hawley for the gift of pT7hD. We are also indebted to Heather Meyers and Carl Ross for technical assistance.

REFERENCES

1. **Baracchini, E., E. Glezer, K. Fish, R. M. Stenberg, J. A. Nelson, and P. Ghazal.** 1992. An isoform varient of the cytomegalovirus immediate-early auto repressor functions as a transcriptional activator. Virology **188:**518–529.
2. **Borrelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus 2 E1A products repress enhancer induced stimulation of transcription. Nature (London) **312:**608–612.
3. **Buratowski, S., and H. Zhou.** 1992. Transcription factor IID mutants defective for interaction with TFIIA. Science **255:**1130–1132.
4. **Chang, C. P., C. L. Malone, and M. F. Stinski.** 1989. A human cytomegalovirus early gene has three inducible promoters that are regulated differentially at various times after infection. J. Virol. **63:**281–290.
5. **Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Horsnell, C. A. Hutchison, T. Kouzarides, J. A. Martignetti, E. Preddie, S. Satchwell, C. P. Tomlinson, K. M. Weston, and B. G. Barrell.** 1990. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. Curr. Top. Microbiol. Immunol. **154:**125–169.
6. **Cheng Kao, C., P. Lieberman, M. Schmidt, Q. Zhou, R. Pei, and A. J. Berk.** 1990. Cloning of a transcriptionally active human TATA binding factor. Science **248:**1646–1650.
7. **Cherrington, J. M., E. L. Khoury, and E. S. Mocarski.** 1991. Human cytomegalovirus *ie2* negatively regulates α gene expression via a short target sequence near the transcription start site. J. Virol. **65:**887–896.
8. **Cherrington, J. M., and E. S. Mocarski.** 1989. Human cytomegalovirus ie1 transactivates the α promoter-enhancer via an 18-base-pair repeat element. J. Virol. **63:**1435–1440.
9. **Depto, A. S., and R. M. Stenberg.** 1992. Functional analysis of the true late human cytomegalovirus pp28 upstream promoter: *cis*-acting elements and viral *trans*-acting proteins necessary for promoter activation. J. Virol. **66:**3241–3246.
10. **Depto, A. S., and R. M. Stenberg.** 1989. Regulated expression of the human cytomegalovirus pp65 gene: octamer sequence in the promoter is required for activation by viral gene products. J. Virol. **63:**1232–1238.
11. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA binding protein that mediate transcriptional activation. Cell **66:**563–576.
12. **Furnari, B. A., E. Poma, T. F. Kowalik, S. Huong, and E. Huang.** 1993. Human cytomegalovirus immediate-early gene 2 protein interacts with itself and with several novel cellular proteins. J. Virol. **67:**4981–4991.
13. **Ghazal, P., and J. A. Nelson.** 1991. Enhancement of RNA polymerase II initiation complexes by a novel DNA control domain downstream from the cap site of the cytomegalovirus major immediate-early promoter. J. Virol. **65:**2299–2307.
14. **Ghazal, P., J. Young, E. Giulietti, C. DeMattei, J. Garcia, R. Gaynor, R. M. Stenberg, and J. A. Nelson.** 1991. A discrete *cis* element in the human immunodeficiency virus long terminal repeat mediates synergistic *trans* activation by cytomegalovirus immediate-early proteins. J. Virol. **65:**6735–6742.
15. **Hagemeier, C., S. Walker, R. Caswell, T. Kouzarides, and J. Sinclair.** 1992. The human cytomegalovirus 80-kilodalton but not the 72-kilodalton immediate-early protein transactivates heterologous promoters in a TATA box-dependent mechanism and directly interacts with TFIID. J. Virol. **66:**4452–4456.
16. **Hermiston, T. W., C. L. Malone, and M. F. Stinski.** 1990. Human cytomegalovirus immediate-early two protein region involved in negative regulation of the major immediate-early promoter. J. Virol. **64:**3532–3536.
17. **Hermiston, T. W., C. L. Malone, P. R. Witte, and M. F. Stinski.** 1987. Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter. J. Virol. **61:**3214–3221.
18. **Horikoshi, M., C. Wang, H. Fujii, J. Cromlish, P. Weil, and R. G. Roeder.** 1989. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature (London) **341:**299–303.
19. **Horikoshi, N., K. Maguire, A. Kralli, E. Maldonado, D. Reinberg, and R. Weinmann.** 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. Proc. Natl. Acad. Sci. USA **88:**5124–5128.
20. **Iwamoto, G. K., M. M. Monick, B. D. Clark, P. E. Auron, M. F. Stinski, and G. W. Hunninghake.** 1990. Modulation of interleukin 1 beta gene expression by the immediate early genes of human cytomegalovirus. J. Clin. Invest. **85:**1853–1857.

20a. **Jupp, R., et al.** Unpublished data.

21. **Jupp, R., S. Hoffmann, A. Depto, R. M. Stenberg, P. Ghazal, and J. A. Nelson.** 1993. Direct interaction of the human cytomegalovirus IE86 protein with the *cis* repression signal does not preclude TBP from binding to the TATA box. J. Virol. **67:**5595–5604.
22. **Jupp, R. A., O. Flores, J. Nelson, and P. Ghazal.** 1993. The 38KDa subunit of human transcription factor IID binds to the TATA box as a multimer. J. Biol. Chem. **268:**16105–16108.
23. **Klucher, K. M., M. Sommer, J. T. Kadonaga, and D. H. Spector.** 1993. In vivo and in vitro analysis of transcription activation mediated by the human cytomegalovirus major immediate-early proteins. Mol. Cell. Biol. **13:**1238–1250.
24. **Lang, D., and T. Stamminger.** 1993. The 86-kilodalton IE-2 protein of human cytomegalovirus is a sequence-specific DNA-binding protein that interacts directly with the negative autoregulatory response element located near the cap site of the enhancer promoter. J. Virol. **67:**323–331.
25. **Lee, D. K., J. Delong, S. Hashimoto, M. Horikoshi, and R. G. Roeder.** 1992. TFIIA induces conformational changes in TFIID via interactions with the basic repeat. Mol. Cell. Biol. **11:**5189–5196.
26. **Lee, W. S., C. C. Kao, G. O. Bryant, X. Liu, and A. J. Berk.** 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. Cell **67:**365–376.
27. **Lieberman, P. M., and A. J. Berk.** 1991. The Zta trans-activator protein stabilizes TFIID association with promoter DNA by direct protein-protein interaction. Genes Dev. **5:**2441–2454.
28. **Lillie, J. W., M. Green, and M. R. Green.** 1986. An adenovirus E1A protein region required for transformation and transcriptional repression. Cell **46:**1043–1051.
29. **Lin, Y. S., and M. R. Green.** 1991. Mechanism of action of an acidic transcription activator in vitro. Cell **64:**971–981.
30. **Liu, B., T. W. Hermiston, and M. F. Stinski.** 1991. A *cis*-acting element in the major immediate-early (IE) promoter of human cytomegalovirus is required for negative regulation by IE2. J. Virol. **65:**897–903.
31. **Liu, F., and M. R. Green.** 1990. A specific member of the ATF transcription factor family can mediate transcription activation by the adenovirus E1a protein. Cell **61:**1217–1224.
32. **Macias, M., and M. F. Stinski.** 1993. An *in vitro* system for human cytomegalovirus immediate early 2 protein (IE2)-mediated site-dependent repression of transcription and direct binding of IE2 to the major immediate-early promoter. Proc. Natl. Acad. Sci. USA **90:**707–711.
33. **Malone, C. L., D. H. Vesole, and M. F. Stinski.** 1990. Transactivation of a human cytomegalovirus early promoter by gene products from the immediate-early gene IE2 and augmentation by IE1: mutational analysis of the viral proteins. J. Virol. **64:**1498–1506.
34. **Nikolov, D. B., S. H. Hu, J. Lin, A. Gasch, A. Hoffmann, M. Horikoshi, N. M. Chau, R. G. Roeder, and S. K. Burley.** 1993. Crystal structure of TFIID TATA box binding protein. Nature (London) **360:**40–46.
35. **Peterson, M. G., N. Tanese, B. Pugh F. and R. Tjian.** 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. Science **248:**1625–1630.
36. **Pizzorno, M. C., and G. S. Hayward.** 1990. The IE2 gene products of human cytomegalovirus specifically down-regulate expression from the major immediate-early promoter through a target sequence located near the cap site. J. Virol. **64:**6154–6165.
37. **Pizzorno, M. C., M. A. Mullen, Y. N. Chang, and G. S. Hayward.** 1991. The functionally active IE2 immediate-early regulatory protein of human cytomegalovirus is an 80-kilodalton polypeptide that contains two distinct activator domains and a duplicated nuclear localization signal. J. Virol. **65:**3839–3852.
38. **Pizzorno, M. C., P. O'Hare, L. Sha, R. L. LaFemina, and G. S.**

Hayward. 1988. *trans*-activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. J. Virol. **62:**1167–1179.

39. **Sambucetti, L. C., J. M. Cherrington, G. W. Wilkinson, and E. S. Mocarski.** 1989. NF-kappa B activation of the cytomegalovirus enhancer is mediated by a viral transactivator and by T cell stimulation. EMBO J. **8:**4251–4258.
40. **Spector, D. H., K. M. Klucher, D. K. Rabert, and D. A. Wright.** 1990. Human cytomegalovirus early gene expression. Curr. Top. Microbiol. Immunol. **154:**21–45.
41. **Staprans, S. I., D. K. Rabert, and D. H. Spector.** 1988. Identification of sequence requirements and *trans*-acting functions necessary for regulated expression of a human cytomegalovirus early gene. J. Virol. **62:**3463–3473.
42. **Stenberg, R. M., A. S. Depto, J. Fortney, and J. A. Nelson.** 1989. Regulated expression of early and late RNAs and proteins from the human cytomegalovirus immediate-early gene region. J. Virol. **63:**2699–2708.
43. **Stenberg, R. M., J. Fortney, S. W. Barlow, B. P. Magrane, J. A. Nelson, and P. Ghazal.** 1990. Promoter-specific *trans* activation and repression by human cytomegalovirus immediate-early proteins involves common and unique protein domains. J. Virol. **64:**1556–1565.
44. **Stenberg, R. M., D. R. Thomsen, and M. F. Stinski.** 1984. Structural analysis of the major immediate-early gene of human cytomegalovirus. J. Virol. **49:**190–199.
45. **Stenberg, R. M., P. R. Witte, and M. F. Stinski.** 1985. Multiple spliced and unspliced transcripts from human cytomegalovirus immediate-early region 2 and evidence for a common initiation site within immediate-early region 1. J. Virol. **56:**665–675.
46. **Stringer, K. F., C. J. Ingles, and J. Greenblatt.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature (London) **345:**783–786.
47. **Tenney, D. J., and P. A. Colberg.** 1991. Human cytomegalovirus UL36-38 and US3 immediate-early genes: temporally regulated expression of nuclear, cytoplasmic, and polysome-associated transcripts during infection. J. Virol. **65:**6724–6734.
48. **Tevethia, M. J., D. J. Spector, K. M. Leisure, and M. F. Stinski.** 1987. Participation of two human cytomegalovirus immediate early gene regions in transcriptional activation of adenovirus promoters. Virology **161:**276–285.
49. **Walker, S., C. Hagemeier, J. G. Sissons, and J. H. Sinclair.** 1992. A 10-base-pair element of the human immunodeficiency virus type 1 long terminal repeat (LTR) is an absolute requirement for transactivation by the human cytomegalovirus 72-kilodalton IE1 protein but can be compensated for by other LTR regions in transactivation by the 80-kilodalton IE2 protein. J. Virol. **66:**1543–1550.
50. **Workman, J. L., R. G. Roeder, and R. E. Kingston.** 1990. An upstream transcription factor, USF (MLTF), facilitates the formation of preinitiation complexes during in vitro chromatin assembly. EMBO J. **9:**1299–1308.
51. **Wu, J., and P. Ghazal.** Unpublished data.