MOLECULAR AND CELLULAR BIOLOGY, June 1996, p. 3085–3093 Vol. 16, No. 6
0270-7306/96/$04.00+0

Interaction of the Viral Activator Protein ICP4 with TFIID through TAF250

MICHAEL J. CARROZZA AND NEAL A. DELUCA*

Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Received 7 February 1996/Returned for modification 13 March 1996/Accepted 1 April 1996

ICP4 of herpes simplex virus is responsible for the activation of viral transcription during infection. It also efficiently activates and represses transcription in vitro depending on the promoter context. The contacts made between ICP4 and the cellular proteins that result in activated transcription have not been identified. The inability of ICP4 to activate transcription with TATA-binding protein in place of TFIID and the requirement for an initiator element for efficient ICP4-activated transcription suggest that coactivators, such as TBP-associated factors, are involved (B. Gu and N. DeLuca, J. Virol. 68:7953–7965, 1994). In this study we showed that ICP4 activates transcription in vitro using an immunopurified TFIID, indicating that TBP-associated factors may be a sufficient subset of coactivators for ICP4-activated transcription. Similar to results seen in vivo, the presence of the ICP4 C-terminal region (amino acids 774 to 1298) was important for activation in vitro. With epitope-tagged ICP4 molecules in immunoaffinity experiments, it was shown that the C-terminal region was also required for ICP4 to interact with TFIID present in a crude transcription factor fraction. In the same assay, ICP4 was unable to interact with the basal transcription factors, TFIIB, TFIIE, TFIIF, and TFIIH and RNA polymerase II. ICP4 could also interact with TBP, independent of the C-terminal region. However, reflective of the interaction between ICP4 and TFIID, the ICP4 C-terminal region was required for an interaction with TAF250-TBP complexes and with TAF250 alone. Therefore, the interfaces or conformation of TBP mediating the interaction between ICP4 and TBP in solution is probably masked when TBP is bound to TAF250. With a series of mutant ICP4 molecules purified from herpes simplex virus-infected cells, it was shown that ICP4 molecules that can bind DNA and interact with TAF250 could activate transcription. Taken together, these results demonstrate that ICP4 interaction with TFIID involves the TAF250 molecule and the C-terminal region of ICP4 and that this interaction is part of the mechanism by which ICP4 activates transcription.

Herpes simplex virus type 1 (HSV-1) encodes approximately 80 transcription units (50, 51). During lytic infection three classes of viral genes, immediate-early, early, and late, are expressed in a temporally regulated manner (39). The immediate-early genes are expressed without prior viral protein synthesis due in part, to the transactivating virion protein VP16 (6, 61). Expression of immediate-early genes allows for the efficient and regulated expression of the remainder of the viral genes (40). Transcription of the viral genes requires the RNA polymerase II transcription machinery of the host (2, 12). One immediate-early gene product, ICP4, serves as the major activator of early and late gene transcription (15, 24, 30, 31, 56, 63) and also as a repressor of its own transcription (15, 57). As a consequence, ICP4 is required for viral gene expression to proceed beyond immediate-early gene expression (14, 20, 62, 80). While genetic and biochemical studies suggest that ICP4 works with the basal transcriptional machinery to activate transcription (3, 10, 32, 34, 35, 42, 76), interactions between ICP4 and specific cellular proteins functioning during promoter activation have not been demonstrated.

ICP4 is a 175-kDa nuclear phosphoprotein (13, 60) that exists as an obligate 350-kDa homodimer (53, 73). Similar to many other transactivators, ICP4 possesses several functional domains, which include a DNA-binding region, nuclear localization region, and two transactivation regions (17, 58, 59, 72). ICP4 specifically binds to DNA having a fairly degenerate consensus sequence (18, 25). While specifically positioned strong ICP4 binding sites mediate repression (34, 47, 54, 65), the role of binding sites in activation is unclear. Activation by ICP4 does not require any single site or collection of sites (9, 22, 23, 37, 75), and its has also been shown that it will activate promoters in vitro without binding sites (32). However, the DNA binding activity of ICP4 appears to be required for activation (59, 72). This activity is not sufficient for repression or activation. In addition to the DNA binding domain, a region amino terminal to amino acid 274 is also required for repression (34). This region and one between amino acids 774 and 1298 allow ICP4 to activate transcription (16, 17, 32, 72). Viruses expressing ICP4 proteins lacking one of these regions are impaired for growth in culture and express reduced levels of viral early and late genes (17, 70). If both regions are deleted, viral growth and early gene expression are severely impaired (73, 76).

It has been shown that ICP4 can form a tripartite complex on DNA with TBP and TFIIB and facilitate the binding of TFIID to the TATA box in the presence of TFIIB (76). While these interactions may be involved in activation, the formation of tripartite complexes has more definitively been shown to mediate repression (34). Little is known about the interaction of ICP4 with cellular proteins during the process of activation. With the use of a reconstituted in vitro transcription system, it has been shown that ICP4 will activate transcription of templates that contain only a TATA box and will activate tran-

* Corresponding author. Mailing address: E1257 Biomedical Science Tower, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261. Phone: (412) 648-9947. Fax: (412) 624-1401.

scription more efficiently with templates that contain a TATA box and an initiator element (32). In addition, genetic studies with recombinant viruses have also implicated the importance of the TATA box (10, 41, 42) and the initiator (11) in the level of ICP4 activation. These results indicate that ICP4 activates transcription through contacts with the basal PolII transcription machinery.

The RNA PolII transcription apparatus is a group of basal or general transcription factors that bind the PolII core promoter region in a sequential order (5). The assembly of a transcription initiation complex begins with the binding of TFIID to the promoter (5, 55). This is followed by the binding of TFIIA, TFIIB (5, 55), TFIIF and RNA PolII (27), and subsequently TFIIE (44), TFIIJ, and TFIIH (26), leading to the accurate initiation of transcription. Recognition of the core promoter by these transcription factors typically depends on the presence of a TATA box, an initiator element, or both. The TATA box is a DNA element located approximately 25 bp upstream of the transcription start site and binds TFIID through a DNA-protein interaction with the TATA-binding protein (TBP) (69), whereas the initiator element overlaps the transcription start site (74). Almost all HSV promoters contain a TATA box. TBP and several other subunits, known as TBP-associated factors (TAFs), constitute what is known as holo-TFIID (21, 82). TAFs have been shown to carry out several functions within the TFIID complex. These functions include promoter selectivity, i.e., recognition of the PolII core promoter (38, 78, 79), recruitment of other basal transcription factors to the initiation complex (66), and coactivator function (7, 21). In vitro studies have shown that TBP, in conjunction with other PolII basal transcription factors, is sufficient for basal levels of transcription; however TAFs, serving as coactivators, are required for activated transcription (4).

It has been previously shown with a reconstituted system and a crude TFIID preparation, that ICP4 can activate transcription but cannot activate transcription when TBP is substituted for TFIID (32). This finding suggests that ICP4 may activate transcription through an interaction with coactivators present in the partially purified TFIID preparation, such as TAFs or other soluble coactivators. In this study we examined whether ICP4, like other transactivators, interacts with TFIID through TAFs to activate transcription. To address this hypothesis we determined if ICP4 can activate in an in vitro transcription system reconstituted with basal transcription factors and immunoaffinity-purified TFIID. It was also determined whether ICP4 can interact in vitro with any of the known basal transcription factors used in the reconstituted in vitro transcription experiments. The results demonstrated that ICP4 activated transcription with immunoaffinity-purified TFIID as efficiently as with a crude TFIID preparation, suggesting that TAFs were a sufficient subset of coactivators to support ICP4-activated transcription. In addition, it was shown that of all the basal transcription factors tested, ICP4 was able to interact only with TFIID. Furthermore, this interaction occurs through the C-terminal region of ICP4, correlating with this region's importance for transactivation. The interaction between ICP4 and TFIID, requiring the C-terminal region of ICP4, was also shown to reflect the interaction between ICP4 and TAF250-TBP complexes and TAF250 alone. The results of this study demonstrate that ICP4 interacts with TFIID involving an interaction with TAF250, which is mediated by the C-terminal region of ICP4, and strongly suggest that this interaction is involved in activation.

MATERIALS AND METHODS

General transcription factors and ICP4. General transcription factors were extracted from HeLa cell nuclei according to the method of Dignam et al. (19). HeLa nuclear extract was fractionated on phosphocellulose (P11) and DEAE-Sephacel (Pharmacia) as described by Reinberg and Roeder (64) and Lin and Green (49), with the modifications described by Gu et al. (35). Designations for transcription factor fractions are as described (34). Fraction AB contains TFIIA, CB contains TFIIE, TFIIF, and TFIIH, CC contains PolII, and DB contains TFIID. TFIID was immunoaffinity purified from α3 HeLa cells containing a hemagglutinin (HA) epitope-tagged TBP, according to the method of Zhou et al. (82). Recombinant human TFIIB (rTFIIB) and recombinant human (rTBP) were purified from *Escherichia coli* by the methods described by Ha et al. (36) and Kao et al. (45), respectively, with the modifications described in Imbalzano et al. (42) and Smith et al. (76). The relative amounts of TBP in the DB fraction and in immunoaffinity-purified TFIID were determined by Western blot (immunoblot) analysis using an antibody directed against TBP (Upstate Biotechnology Inc.) and comparing the resulting signals to those from known amounts of purified rTBP.

Wild-type (wt) and mutant ICP4s were purified from Vero cell nuclei infected with HSV expressing wt and mutant forms of ICP4 described previously (43, 71). The X25 ICP4 mutant was purified from the nuclei of the HSV-infected X25 cells as described by Shepard et al. (73).

TAF250 was prepared by infecting Sf9 cells with an HA epitope-tagged TAF250 expressing baculovirus (67). At 40 h postinfection cells were scraped, washed in Tris-buffered saline (TBS), and washed in RSB buffer (10 mM NaCl, 10 mM Tris-HCl [pH 7.4], 3 mM $MgCl_2$, 0.1 mM TLCK, [*N*-α-*p*-tosyl-L-lysine chloromethylketone], 0.1 mM phenylmethylsulfonyl fluoride [PMSF]). The cells were swollen for 10 min in RSB containing 0.1% Nonidet P-40 P-40 and disrupted with a Dounce homogenizer. The isolated nuclei were extracted for 45 min in buffer containing 50 mM Tris-HCl (pH 8.0), 0.5 M KCl, 2% Nonidet P-40, 0.2 mM TLCK, 0.1 mM PMSF. The extract was clarified by centrifugation in a Beckman SW50.1 rotor for 1 h at 45,000 rpm at 4°C. The clarified extract was applied to a preparative Superose 6 column and chromatographed in CB (0.5) buffer containing 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 M KCl, 0.01% CHAPS {3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate}, 10 mM β-mercaptoethanol, 0.1 mM TLCK, and 0.1 mM PMSF. TAF250 fractions were identified by dot blot analysis of fractions with the HA epitope-recognizing monoclonal antibody 12CA5 (Boehringer Mannheim). TAF250 eluted just after the void. While not homogeneous, TAF250 was the major protein in this preparation as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

In vitro transcription assays. Transcription reaction mixtures consisted of 0.5 μl of AB (TFIIA); 2 μl of CB (TFIIE, TFIIF, or TFIIH); 4 μl of CC (PolII); 0.5 μl of rTFIIB; either 4 μl of DB (TFIID), 1 μl of immunoaffinity-purified TFIID, or 0.5 μl of rTBP; and the indicated amount of wt ICP4 or mutant ICP4 protein. In addition, each reaction mixture contained 40 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [pH 7.9], 60 mM KCl, 12% glycerol, 8.3 mM $MgCl_2$, 0.6 mM ribonucleoside triphosphates, 12 U of RNasin, 0.3 mM dithiothreitol, and 10 fmol of template DNA in a final volume of 30 μl. The template contains the HSV glycoprotein C promoter, which consists of a TATA box and initiator element (Fig. 1A). Reactions were carried out at 30°C for 1 h. The reactions were stopped with the addition of 70 μl of transcription stop buffer (150 mM sodium acetate, 15 mM EDTA, 150 μg of tRNA per ml), and then the mixtures were subjected to phenol extraction and ethanol precipitation. The RNA pellet was resuspended in 10 μl of buffer containing 10 mM Tris-HCl (pH 7.5), 250 mM KCl, 1 mM EDTA, and 3 ng of 5′ ^{32}P-end-labeled primer. This primer is an oligonucleotide complementary to nucleotides +144 to +174 downstream of the gC transcription start site (Fig. 1A).

The annealing mixture was heated for 30 min at 65°C and then allowed to cool slowly to room temperature. Primer extension was performed on the samples in a solution containing 50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM deoxynucleoside triphosphates, 12 U of RNasin, 50 μg of actinomycin D per ml, and 300 U of Moloney murine leukemia virus reverse transcriptase (Gibco/BRL), in a final volume of 40 μl for 1 h at 42°C. The addition of 60 μl of primer extension stop buffer (1 M ammonium acetate, 20 mM EDTA) was followed by phenol extraction and ethanol precipitation. The pellets were resuspended in 5 μl of formamide loading buffer (96% formamide, 0.005% bromophenol blue-xylene cyanol, 10 mM EDTA, 10 mM NaOH), heated for 5 min at 95°C, quickly cooled on ice, and loaded onto 6% polyacrylamide denaturing sequencing gels. The gels were dried and exposed to Amersham Hyperfilm.

Construction of HA epitope-tagged wt and n208 ICP4 viruses. An oligonucleotide encoding the HA epitope (YPYDVPDYAV) was inserted into the ICP4 nonsense mutant n208 (Fig. 2A) coding sequence at an in frame *Pst*I linker insertion within plasmid pi3 (71). The resulting plasmid, peICP4 was used to rescue the ICP4 nonsense mutant n12 as described previously (17). Epitope-tagged wt ICP4 and n208 recombinants were propagated on Vero and E5 cells (16), respectively. Plaque isolates were screened by Southern analysis. Viruses producing epitope-tagged ICP4 were plaque purified twice more and again screened by Southern blot hybridization. Stocks of the epitope-tagged wt (*e*wt)

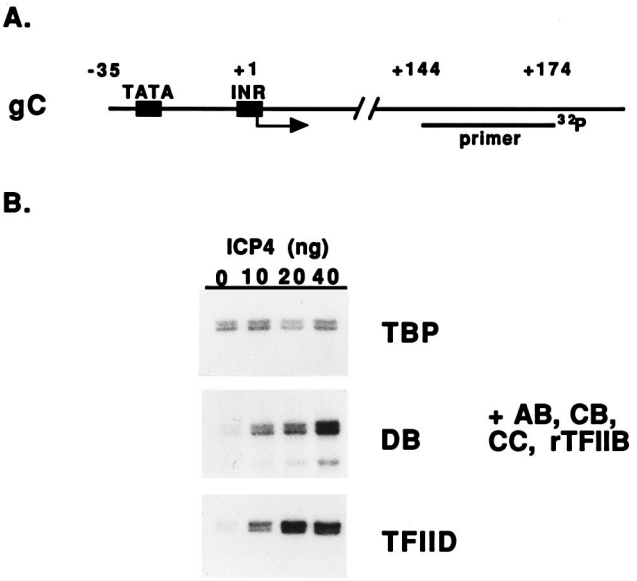

FIG. 1. ICP4 efficiently activates transcription with immunoaffinity-purified TFIID. (A) Schematic representation of HSV gC promoter used as a template for in vitro transcription. The gC TATA box is located 25 bp upstream of transcription start site (indicated by arrow), while the Inr overlaps the start site. The initiator sequence (INR) was previously defined and is similar to the TdT initiator (32). The primer used for primer extension analysis of in vitro transcripts is complementary to the region between +144 and +174 of the template. (B) In vitro transcription reconstituted with basal factors and either rTBP, TFIID from the HeLa DB fraction, or immunoaffinity-purified TFIID and different concentrations of ICP4. The relative amount of TFIID or TBP added to the reaction mixture was determined by Western analysis of each preparation for TBP. The amounts of TBP, DB, and TFIID added to each reaction mixture varied depending on their basal transcription activity; rTBP reaction mixtures contained 10 ng of TBP, DB reaction mixtures contained 0.5 ng of TBP equivalents, and TFIID reaction mixtures contained 0.3 ng of TBP equivalents. When no TBP-containing preparation was added, transcription was not observed (data not shown). For each set of samples, the indicated quantities of ICP4 were added. The first sample for each set did not receive any ICP4 and represents basal levels of transcription. Following primer extension of the in vitro transcription products, the 174-nucleotide reverse transcripts were run on 6% sequencing gels, dried, and exposed to X-ray film. A longer exposure time for the TBP reactions is shown to clearly represent that no activation by ICP4 had occurred.

and n208 (*e*n208) ICP4 virus were prepared on Vero and E5 cells, respectively, and ICP4 proteins were purified as described above.

***e*wt and *e*n208 ICP4 immunoaffinity assay.** Approximately 1 μg of *e*wt and *e*n208 ICP4 in 200 μl of D.2MN buffer (20 mM HEPES [pH 7.9], 10% glycerol, 200 mM KCl, 2 mM EDTA, 1 mM dithiothreitol, 0.5 mM PMSF, 0.1% Nonidet P-40, 12.5 mM $MgCl_2$) and 200 μl of D.2MN alone were each incubated with 10 μl of 12CA5 covalently coupled to protein A-Sepharose (82) for 3 h at 4°C with gentle mixing. *e*wt-, *e*n208-, and 12CA5-Sepharose were washed twice in 0.5 ml of D.1MN (D.2MN with 100 mM KCl instead of 200 mM KCl). To the washed Sepharose either 18 ng of rTBP, 200 ng of rTFIIB, 25 μl of CC or DB, or 50 μl of the CB fraction in D.1MN was added. The samples were incubated for 1 h at room temperature with gentle mixing and washed five times in 1 ml of D.1MN. The HA peptide (10 μl; Babco) (2 mg/ml) in D.3MN (D.2MN with 300 instead of 200 mM KCl) was added to the Sepharose, and the mixture was incubated for 1 h at room temperature with gentle mixing. Eluants were run on SDS–8% PAGE gels and transferred to polyvinylidene difluoride membranes. Western analysis of the filters was performed with antibodies directed against TBP and TFIIB, TFIIE, and TFIIF (Upstate Biotechnology Inc.), PolII (Promega), and ICP4 (13) (provided by Richard Courtney, The Pennsylvania State University) and visualized by chemiluminescence (Boehringer Mannheim).

TAF250 immunoaffinity assays. The *e*TAF250 Superose 6 peak fraction (50 μl) was diluted to 100 mM KCl with 200 μl of D.0MN (D.2MN with no KCl instead of 200 mM KCl). 12CA5-Sepharose (10 μl) was incubated with either the diluted *e*TAF250 or D.1MN for 3 h at 4°C with gentle mixing. The TAF250-bound Sepharose and 12CA5-Sepharose control were washed twice with 0.5 ml of D.1MN. To the TAF250 and control Sepharose, either 100 ng of TBP or 50 ng of purified wt or mutant ICP4 protein in D.1MN was added. Binding reactions were carried out for 1 h at room temperature with gentle mixing. The samples were then washed five times in D.1MN. In the experiment involving the TAF250-TBP complexes, the ICP4 binding step was performed as described above following the TBP binding and washings steps. Bound proteins were eluted by adding 10 μl of the HA peptide (2 mg/ml) in D.3MN to each sample and incubating the mixtures for 1 h at room temperature with gentle mixing. The eluants were run on an SDS–8% PAGE gel. Proteins were then transferred to polyvinylidene difluoride membranes and subjected to Western analysis with antibodies directed against ICP4 and TBP. Signals on Western blots were visualized by chemiluminescence.

RESULTS

ICP4 activates early and late gene promoters during the course of HSV infection. Experiments using nuclear extracts (3) or reconstituted transcription factor preparations (32) have shown that ICP4 also activates transcription in vitro. The studies with reconstituted transcription involved the use of a TFIID preparation that was fractionated through phosphocellulose and DEAE-Sephacel (DB). That ICP4 could activate transcription with this TFIID preparation and not recombinant TBP reflects the requirement for TAFs associated with TBP and/or soluble coactivators. In this study we examined the ability of ICP4 to activate transcription, using immunoaffinity-purified TFIID along with the remaining basal transcription factors. We also investigated the ability of ICP4 to interact with TFIID and other basal transcription factors present in the reconstituted system that allowed activated transcription.

ICP4 activates transcription with immunoaffinity-purified TFIID. The gC promoter template used in this study is shown in Fig. 1. This promoter contains both a TATA box and an initiator element. Both these elements have been shown to be required for optimal levels of activated transcription by ICP4 in vitro (32). In vitro transcription reactions were performed to assess the ability of ICP4 to activate transcription with either rTBP, a crude TFIID preparation (DB), or immunoaffinity-purified TFIID, along with the indicated basal transcription factor preparations. The reaction products were visualized by primer extension with the ^{32}P-end-labeled primer complementary to nucleotides +144 to +174 of the gC RNA transcript (Fig. 1A). The resulting 174-nucleotide reverse transcripts were run on sequencing gels, dried, and exposed to X-ray film.

Consistent with previous observations (32), TBP was unable to support ICP4 activated transcription (Fig. 1B, top panel); however, when the fractionated TFIID preparation (DB) was used, ICP4 efficiently activated transcription (Fig. 1B, middle panel). This indicates that coactivators present in the DB preparation along with TBP are required for activation. When immunoaffinity-purified TFIID was used, ICP4 activated transcription to levels comparable to those seen with the DB preparation (Fig. 1B, bottom panel). This result indicates that TAFs, or proteins tightly associated with TFIID, are a sufficient subset of coactivators to support ICP4-activated transcription. In contrast, Gal4-VP16 is unable to activate even when the DB preparation is used (29, 33), suggesting that coactivators not associated with TFIID are also required.

The ICP4 C-terminal region is important for activated transcription in vitro. Various mutant ICP4 proteins were purified from the nuclei of Vero cells infected with the appropriate ICP4 mutant virus and used in the in vitro transcription reactions. The primary structures of these proteins relative to the wt protein are depicted in Fig. 2A. The mutant i13 contains a Gly-Cys-Ser insertion mutation at amino acid 338 within the DNA binding domain that impairs its DNA binding activity, its ability to transactivate viral gene expression in vivo and in transient assays, and its ability to support viral growth (71, 72). Mutant d8-10 contains a deletion removing amino acids 142 to 210, marginally reducing the ability of ICP4 to activate transcription in cells (72, 81). Mutant n208 contains a nonsense

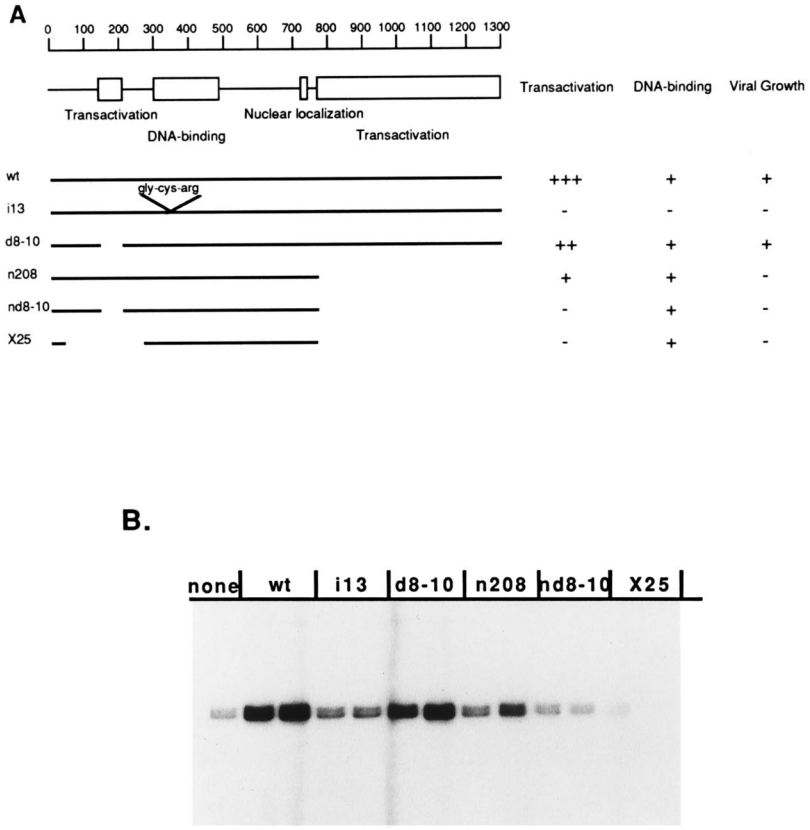

FIG. 2. In vitro transcription with purified mutant ICP4 proteins. (A) Domain map of ICP4 and properties of mutants relative to wt ICP4. The important regions of ICP4 are indicated by boxes, with their amino acid locations indicated by the scale above the boxes. The primary structure of each of the mutant molecules relative to the wt protein is indicated. To the right of each mutant diagram the in vivo transactivation properties, DNA-binding properties, and the growth properties of viruses expressing these proteins are indicated. Transactivation in vivo was determined by transient transfection assays and/or the transcription rates of selected viral genes in the context of viral infection. Degrees of transactivation are graded: +++, wt levels; ++, moderate activation; +, weak activation; and −, no activation. DNA-binding was determined by electrophoretic mobility shift assay. This summary is derived from several previous studies (17, 58, 59, 72). (B) In vitro transcription analysis of wt and mutant ICP4s. In vitro transcription reactions were carried out as described in the legend to Fig. 1. Each reaction mixture contained 0.5 ng of TBP equivalents of DB as a source of TFIID. Each ICP4 protein was tested at 20 and 40 ng per reaction as determined by Western blot analysis of each mutant protein normalized to the wt. The lane labeled none corresponds to a control with no ICP4 and represents basal levels of transcription.

mutation at amino acid 774, severely impairing its ability to activate transcription in vivo and to support viral growth (16, 17). Mutant nd8-10 contains both the d8-10 and n208 mutations. This mutant binds DNA but does not activate gene expression in vivo or support viral growth (76). In mutant X25 both transactivation regions are deleted. Mutant X25 differs from nd8-10 in that amino acids 31 to 274 are deleted. This mutant also binds DNA but is unable to activate transcription in vivo or to support viral growth (73).

As expected, the intact (wt) ICP4 efficiently activated transcription (Fig. 2B). The deletion mutant d8-10 activated transcription only slightly less efficiently than the wt. i13 was severely impaired for its ability to activate transcription because of its lack of DNA binding activity. The truncated mutant n208 (Fig. 2A) activated transcription quite poorly, and the mutants nd8-10 and X25, lacking both transactivation regions, produced only basal levels of transcription. Interestingly, mutant X25 actually caused a reduction in basal transcription levels, suggesting that this protein in some way inhibits the basal transcription machinery. Therefore, provided that the DNA-binding domain of ICP4 is intact, the C-terminal region of ICP4 is a major contributor to the ability of ICP4 to activate transcription in vitro. This is consistent with results obtained in vivo.

The C-terminal region of ICP4 interacts with TFIID. On the basis of the ability of ICP4 to efficiently activate transcription in the reconstituted system, we determined whether ICP4 can bind any of the basal transcription factors present in the fractions and if any of the regions of ICP4 that were important for activation were also important for the observed interaction(s) with the basal factors. In order to address this question we used an immunoaffinity assay with purified HA epitope-tagged wt (*e*wt) and n208 (*e*n208) ICP4 proteins. These proteins were made by inserting the epitope DNA sequence into the ICP4 coding sequence and recombining this construct into the viral ICP4 loci by homologous recombination. Recombinant viruses were isolated and used to infect Vero cells, and the expressed *e*wt or *e*n208 ICP4 proteins were purified as described in Materials and Methods. The ewt or en208 proteins were then bound to covalently-coupled 12CA5-sepharose. ICP4 (wt and n208)-Sepharose was then incubated with either rTBP, rTFIIB, or the transcription factor fractions CC, CB, or DB, washed, and eluted with the HA peptide. The TFIIA fraction (AB) was not tested since it does not seem to greatly contribute to basal or activated transcription in our system (32). The proteins present in transcription factor preparations used in these experiments are shown in Coomassie blue-stained gels (Fig. 3A). The rTBP and rTFIIB preparations are very homogeneous,

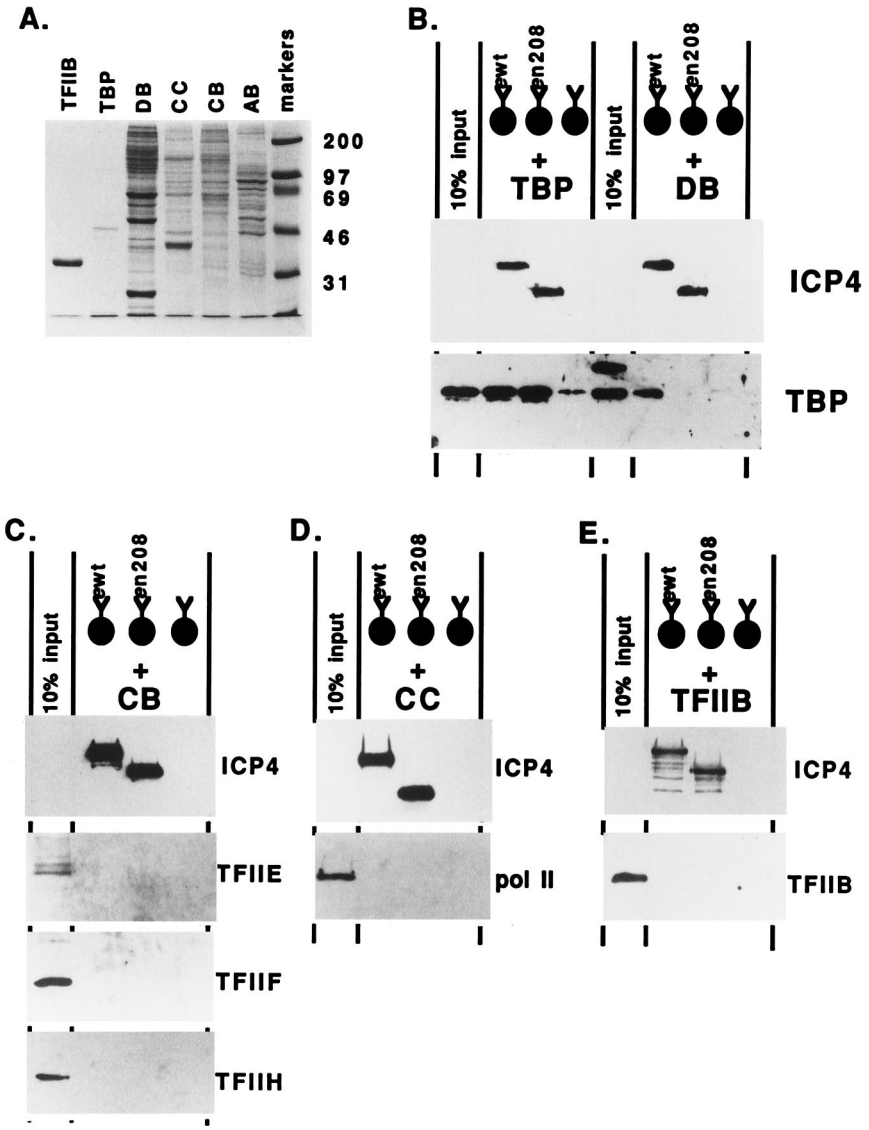

FIG. 3. Binding of basal transcription factors to ICP4. (A) Coomassie blue-stained SDS–10% PAGE of basal transcription factor preparations used in the in vitro transcription analysis. The positions of molecular weight markers (in thousands) are indicated on the right. (B) Binding of rTBP and TFIID to *e*wt and *e*n208 ICP4. rTBP or the DB fraction was incubated with either *e*wt or *e*n208 bound to 12CA5-Sepharose or as a control with 12CA5-Sepharose. The bound proteins were eluted with the HA peptide and run on SDS–8% PAGE gels along with 10% of the input TBP and DB. The gel was subjected to Western analysis for TBP. To control for equal binding and elution of the ICP4 proteins, the filters were stripped and reprobed for ICP4. (C) Binding of TFIIE, TFIIF, and TFIIH to *e*wt and *e*n208 ICP4. The CB fraction was incubated with the *e*wt- or *e*n208-bound Sepharose and the 12CA5-Sepharose negative control. Eluted samples along with 10% of the input CB fraction were run on SDS–10% PAGE gels and subjected to Western blot analysis for the 56-kDa subunit of TFIIE, the 30-kDa subunit of TFIIF, the 62-kDa subunit of TFIIH, and ICP4. (D) Binding of PolII to *e*wt and *e*n208 ICP4. The CC fraction was incubated with the *e*wt- or *e*n208-bound Sepharose and the 12CA5-Sepharose negative control. Eluted samples along with 10% of the input CC fraction were run on SDS–7.5% PAGE gels and subjected to Western blot analysis for the PolII large subunit and ICP4. (E) Binding of rTFIIB to *e*wt and *e*n208 ICP4. rTFIIB was incubated with *e*wt- or *e*n208-bound Sepharose and the 12CA5-sepharose negative control. Eluted samples were run on SDS–10% PAGE gels and subjected to Western blot analysis for TFIIB and ICP4.

whereas the transcription factor fractions obtained from the fractionation of HeLa cells are very heterogeneous. As a control, the protein preparations were also incubated with 12CA5-Sepharose, which did not receive any ICP4 protein, and subjected to the same experimental conditions. After elution of the bound protein with the HA peptide, the eluants were subjected to Western analysis with antibodies directed against the various general transcription factors.

In this assay, both wt and n208 ICP4 bound TBP, while only wt ICP4 bound TFIID (Fig. 3B). The lower mobility band seen in the 10% DB input represents cross-reactivity with the TBP antibody and an unknown protein in the complex DB fraction. However, only the 46-kDa TBP band is enriched after binding to ICP4. This result indicates that ICP4 can interact with TFIID through an activity provided by its C-terminal region. In addition, the observation that n208 bound free TBP, but not TFIID, suggests that the interface on TBP for interacting with ICP4 might be masked within the TFIID complex. When the basal transcription factors TFIIE, TFIIF, and TFIIH in the CB fraction were tested, none was able to bind ICP4 (Fig. 3C). Likewise, PolII present in the CC fraction and rTFIIB were also unable to bind ICP4 (Fig. 3D and E, respectively). In each case, equivalent amounts of *e*wt and *e*n208 ICP4 were present as indicated by the Western blots probed for ICP4 (Fig. 3B to

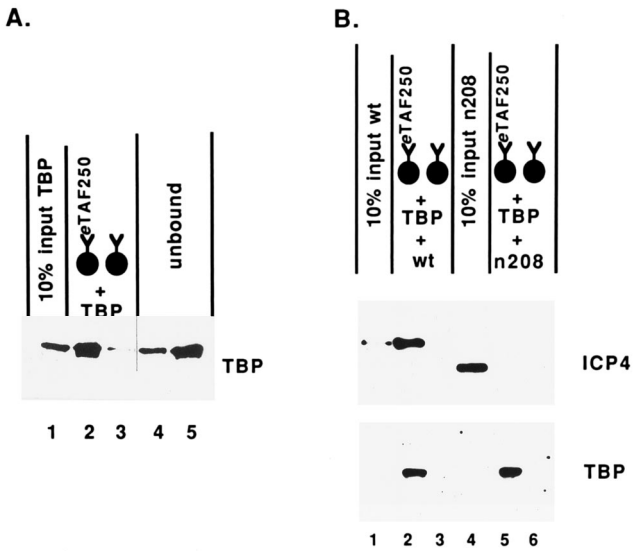

FIG. 4. Binding of ICP4 to TAF250-TBP complexes. (A) Binding of TBP to TAF250. *r*TBP was incubated with *e*TAF250-bound Sepharose and 12CA5-Sepharose as a negative control. HA peptide-eluted samples, along with 10% of the input *r*TBP, and the post-TBP binding supernatants were run on SDS–8% PAGE gels and subjected to Western blot analysis for TBP. (B) Binding of ICP4 to TAF250-TBP. Purified wt and n208 ICP4 proteins were incubated with TAF250-TBP-Sepharose and 12CA5-Sepharose as a negative control. The HA peptide-eluted samples and 10% of the input wt and n208 ICP4 proteins were run on SDS–8% PAGE and subjected to Western blot analyses for ICP4 (top panel) and for TBP (bottom panel).

E, top panels). From the results of this experiment, it appears that ICP4 has the greatest affinity for TFIID and that this interaction requires the C-terminal region of ICP4.

The C-terminal region of ICP4 is required for interaction with TAF250-TBP complexes. On the basis of the observation that ICP4 interacted with TFIID through its C-terminal region, we next wanted to determine whether ICP4 could interact with a TAF250-TBP complex and whether the C-terminal region was involved. It is known that TBP associates with the TFIID complex through an interaction with TAF250. Accordingly, TBP was bound in saturating amounts to *e*TAF250 immobilized on 12CA5-Sepharose. This step was followed by incubation of *e*TAF250-TBP-Sepharose with untagged wt and n208 ICP4. Following binding and elution with the HA peptide, the samples were subjected to Western blot analysis for ICP4. As negative controls, TBP and subsequently wt and n208 ICP4 were also incubated on 12CA5-Sepharose without the addition of TAF250. The amount of ICP4 that bound to TAF250-TBP-Sepharose was determined by Western blot analysis of the material eluted with the HA peptide.

To insure that TBP bound to the immobilized TAF250, TBP was incubated with the TAF250-Sepharose, washed, and eluted with the HA peptide. As shown in Fig. 4A, lane 2, TBP eluted with the peptide, and its presence in the eluant was dependent on the prior addition of TAF250 (Fig. 4A, lanes 2 and 3). In addition, gel filtration analysis of the eluant depicted in lane 2 indicated that all the TBP in the eluted sample was associated with TAF250 (data not shown). The reduced, albeit continued presence of TBP in the postbinding supernatant from the TAF250-Sepharose (lane 4), relative to the Sepharose sample (lane 5), indicates that the TAF250 was saturated for TBP. The addition of more TBP to the binding reaction mixture did not result in an increase in bound TBP (data not shown). The TAF250-TBP-Sepharose beads were then used as an affinity matrix for ICP4. As shown in Fig. 4B, lanes 2 and 5, wt ICP4 bound the partial complex, whereas n208 did not. The lower panel of Fig. 4B (lanes 2 and 5) demonstrates that the partial complex was present in equivalent amounts for both the wt and n208 samples as indicated by the similar amounts of TBP in the eluants of both samples. These results show that the C-terminal region of ICP4 allows it to interact with a complex consisting of only TAF250 and TBP. Therefore, the pattern of interaction seen between TAF250-TBP and wt and n208 ICP4 molecules reflected the pattern of interactions seen between TFIID and wt and n208 ICP4 molecules (Fig. 3A). Furthermore, the ability of n208 to interact with TBP (Fig. 3A), and not TFIID or TAF250-TBP, indicates that the structure of TBP responsible for the interaction with n208, and probably wt ICP4, is masked by TAF250.

The C-terminal region of ICP4 is required for interaction with TAF250. To determine if the interaction between ICP4 and TAF250-TBP is a function of interaction with TAF250 and if the C-terminal region of ICP4 is required, immunoaffinity experiments were performed with TAF250 alone and the various purified ICP4 mutants described in Fig. 2A. TAF250 was immobilized on 12CA5-Sepharose, incubated with the wt or mutant ICP4 proteins, eluted with the HA peptide, and subjected to Western blot analysis for ICP4. To control for nonspecific binding of ICP4 to 12CA5-Sepharose, ICP4 was also incubated with Sepharose without the addition of TAF250. As a positive control, the experiment was also performed with TBP.

As expected, the TBP bound to TAF250 (Fig. 5). Like TBP, wt ICP4 also bound to TAF250. Mutants i13 and d8-10, both of which contain the C-terminal region, were also able to bind TAF250. However, mutants n208, nd8-10, and X25, all of which lack the C-terminal region, were unable to bind to TAF250. These results demonstrate that ICP4 interacts with TAF250 and that the C-terminal region of ICP4 is required for this interaction. This finding suggests that the interaction between ICP4 and TAF250-TBP complexes is mainly due to ICP4-TAF250 interactions.

DISCUSSION

ICP4 is a very large protein that may possess multiple interfaces for interacting with cellular transcription factors to carry out various aspects of its function in the regulation of viral gene expression. Numerous previous studies have shown that multiple regions of the protein, in addition to its DNA binding domain, affect its ability to activate and repress transcription (17, 32, 34, 58, 72). The region that has the greatest effect on the ability of ICP4 to activate transcription is that between amino acids 774 and 1298. An ICP4 molecule lacking this region (n208) can still form dimers, bind to DNA, repress transcription of its own promoter, and marginally activate some promoters (16, 17, 70). In this study we demonstrate that immobilized ICP4 can be used as an affinity matrix to enrich for TFIID in a complex mixture of proteins and that this interaction is dependent on the region of ICP4 between amino acids 774 and 1298. Moreover, the C-terminal region of ICP4 was also required to interact with a TAF250-TBP complex and TAF250 alone. Therefore, it appears that ICP4 interacts with TFIID through interaction with TAF250 and that the C-terminal region of ICP4 mediates this interaction.

Functional interaction of ICP4 with TFIID. Previous genetic studies have shown that ICP4 activates promoters with TATA boxes with a low affinity for TBP more efficiently than those with a high affinity for TBP (10, 42). Additionally, the results of kinetic experiments have suggested that the pseudorabies

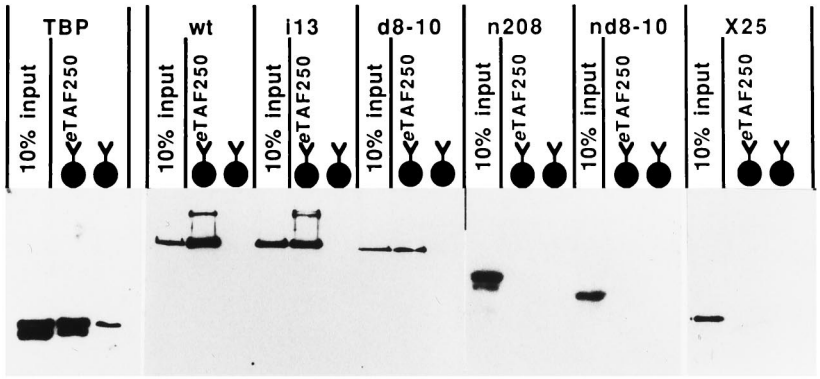

FIG. 5. Binding of wt and mutant ICP4 proteins to TAF250. Purified wt and mutant ICP4 proteins and rTBP as a positive control were incubated with *e*TAF250-bound Sepharose and 12CA5-Sepharose as a negative control. The mutant ICP4 proteins are described in Fig. 2A. The HA peptide-eluted samples and 10% of the input rTBP and ICP4 proteins were run on SDS–8% PAGE and subjected to Western blot analyses for TBP and ICP4.

counterpart of ICP4 can facilitate the functioning of TFIID (1). It has also been shown that a crude TFIID fraction along with the other factors was sufficient to allow ICP4 to activate transcription (32). Despite these findings, an interaction between ICP4 and TFIID has not been previously demonstrated. Moreover, many activators will not efficiently activate transcription with immunoaffinity-purified TFIID (29, 48). In such cases, the USA fraction, which contains among other proteins, the p15 or PC4 coactivator, is needed along with TFIID to activate transcription (29, 46, 52). In this study we show that ICP4 interacts with TFIID and that immunoaffinity-purified TFIID works as well as less-pure TFIID preparations to activate transcription. In this study, we found only p15 in the flowthrough of the DEAE column on the P11 D fraction (data not shown). This finding is consistent with previously published reports (29, 46). Interestingly, the amino-terminal region of ICP4 has amino acid similarity to p15 (46). The possession of this region may help obviate the need for coactivators such as p15.

We also found that wt ICP4 and the C-terminal deletion mutant n208 interacted with TBP using our assay, whereas only wt ICP4 interacted with TFIID using the same assay. These results suggest that the interface(s) or conformation of TBP needed for the interaction with n208 is masked or altered when part of the TFIID complex. This same pattern of interaction was seen when TBP was bound to TAF250, suggesting that the interaction between TBP and TAF250 obviates the interaction between the n208 molecule and TBP. In contrast, we have previously shown that the amino terminus of ICP4 along with the DNA binding domain allowed ICP4 to form a tripartite complex on DNA along with TBP and TFIIB (34). However, this complex mainly occurs only when a strong ICP4 binding site is positioned in the proper orientation and no more than 40 bp away from the TATA box (34, 47). Several studies have shown that this structure is involved in the repression of transcription by ICP4 (34, 47, 68). The formation of the structure requires DNA binding sites for both TBP and ICP4 and is accompanied by a severe distortion of the DNA at both the ICP4 binding site and the TATA box (47). It is possible that the distortion of the DNA, along with interactions between ICP4 and complexes bound at the TATA box, may alter the conformation of the complex, allowing contacts with TBP. Such a conformational change may be part of the mechanism of repression. Consistent with this hypothesis is the observation that ICP4 does not repress basal transcription but rather reduces the efficiency of a variety of activators (34, 35). n208 is as active a repressor as wt ICP4 in vitro and elicits all of the activities described above (34, 76). However, it does not activate transcription nearly as well as wt ICP4 (Fig. 2). While it is possible that some events or interactions involved in repression are also involved in activation, some interactions involved in activation are likely not to be involved in repression. These interactions are likely to be determined by the carboxy terminus of ICP4.

Role of the C terminus of ICP4 in activation. The importance of the carboxy-terminal region of ICP4 to its function was first indicated by observations of the extensive amino acid conservation within this region among the ICP4 analogs of several alpha-herpesviruses (8, 80). Studies with cell cultures demonstrated that this region is necessary for efficient activation (16, 17). The results presented in this study demonstrate that the C-terminal region of ICP4 is important for interacting with TFIID and that this correlates with the importance of this region in activating transcription in vitro (Fig. 2B). The interaction between ICP4 and TFIID reflects the interaction between ICP4 and TAF250, suggesting that at least part of the interaction between ICP4 and TFIID is mediated by an interaction between ICP4 and TAF250.

This study demonstrates that the C-terminal 524 amino acids of ICP4 are required for the interactions with TFIID and TAF250. Given the large size of this region, it is possible that these interactions represent a subset of activities specified by this region. Attempts to narrow the functional regions of the C-terminal region of the protein have not been very fruitful to date, presumably because of conformational constraints. It has not been possible to express stable or soluble ICP4 molecules containing only the C terminus without the dimerization domain, which is located in the amino-terminal region of the molecule (28). On the other hand, ICP4 molecules lacking the C-terminal region are stable dimers in solution, bind specifically to DNA, repress transcription as well as the wt, form tripartite complexes with TBP and TFIIB, and marginally activate transcription. The possibility that the C-terminal region contacts the amino-terminal region is suggested by studies showing that the C terminus can complement defects in the amino terminus through the formation of heterodimers (70). Thus, while the C-terminal region is required for interaction with TAF250 and TFIID, it is possible that a tertiary or quaternary structure requiring the C-terminal region and the N-terminal region forms the interface for this interaction.

In this report, we demonstrate that similar to other TAFs possessing coactivator activity, TAF250 can interact with the

transactivator ICP4. Since ICP4 is a large molecule that potentially possesses multiple interfaces for making contacts with other proteins, it is likely that contact with TAF250 is not the only interaction involved in ICP4 activation. Instances of transactivators participating in multiple TAF interactions are not unprecedented. It has been shown that p53 requires an interaction with both TAF40 and TAF60 to activate transcription (77). Studies have also shown that TAF150 and TAF110 are required for Sp1 activation (7). TAF150, along with TAF250 and TBP, is required for optimal levels of basal transcription from TATA/Inr promoters (78). Our previous studies have shown that a functional Inr region greatly facilitates activation by ICP4 in vitro (32) and in viral infection (11). It is possible that contacts with TAF250 and an Inr-binding protein such as TAF150 (78, 79) are involved in activation by ICP4. Additional studies focusing on the interaction between ICP4 and holo-TFIID are in progress and may shed light on this possibility.

ACKNOWLEDGMENTS

We thank Baohua Gu for helpful discussions and comments on the manuscript.

This work was supported by an NIH grant, AI30612, to N.A.D.

REFERENCES

1. **Abmayr, S. M., J. L. Workman, and R. G. Roeder.** 1988. The pseudorabies immediate early protein stimulates in vitro transcription by facilitating TFIID: promoter interactions. Genes Dev. **2:**542–553.
2. **Alwine, J., W. Stein, and C. Hill.** 1974. Transcription of herpes simplex type 1 DNA in nuclei isolated from infected Hep-2 and KB cells. Virology **60:** 302–307.
3. **Beard, P., S. Faber, K. W. Wilcox, and L. I. Pizer.** 1986. Herpes simplex virus immediate early infected-cell polypeptide 4 binds to DNA and promotes transcription. Proc. Natl. Acad. Sci. USA **83:**4016–4020.
4. **Buratowski, S.** 1995. Mechanisms of gene activation. Science **270:**1773–1774.
5. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56:** 549–561.
6. **Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for the stimulation of immediate early transcription. J. Mol. Biol. **180:**1–19.
7. **Chen, J.-L., L. Donatella Attardi, C. P. Verrijzer, K. Yokomori, and R. Tjian.** 1994. Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell **79:**93–105.
8. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17:**4637–4646.
9. **Coen, D. M., S. P. Weinheimer, and S. L. McKnight.** 1986. A genetic approach to promoter recognition during trans induction of viral gene expression. Science **234:**53–59.
10. **Cook, W. J., B. Gu, N. A. DeLuca, E. B. Moynihan, and D. M. Coen.** 1995. Induction of transcription by a viral regulatory protein depends on the relative strengths of functional TATA boxes. Mol. Cell. Biol. **15:**4998–5006.
11. **Cook, W. J., S. M. Lin, N. DeLuca, and D. M. Coen.** 1995. Initiator elements and regulated expression of the thymidine kinase gene. J. Virol. **69:**7291–7294.
12. **Costanzo, F. G. Campadelli-Fiume, L. Foà-Tomasi, and E. Cassai.** 1977. Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. J. Virol. **21:**996–1001.
13. **Courtney, R. J., and M. Benyesh-Melnick.** 1974. Isolation and characterization of a large molecular weight polypeptide of herpes simplex virus type 1. Virology **62:**539–551.
14. **DeLuca, N. A., A. McCarthy, and P. A. Schaffer.** 1985. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate-early regulatory protein ICP4. J. Virol. **56:**558–570.
15. **DeLuca, N. A., and P. A. Schaffer.** 1985. Activation of immediate-early, early, and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. Mol. Cell. Biol. **5:**1997–2008.
16. **DeLuca, N. A., and P. A. Schaffer.** 1987. Activities of herpes simplex virus type 1 (HSV-1) ICP4 genes specifying nonsense peptides. Nucleic Acids Res. **15:**4491–4511.
17. **DeLuca, N. A., and P. A. Schaffer.** 1988. Physical and functional domains of the herpes simplex virus transcriptional regulatory protein ICP4. J. Virol. **62:**732–743.
18. **DiDonato, J. A., J. R. Spitzner, and M. T. Muller.** 1991. A predictive model for DNA recognition by the herpes simplex virus protein ICP4. J. Mol. Biol. **219:**451–470.
19. **Digman J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription by RNA polymerase II in soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
20. **Dixon, R. A. F., and P. A. Schaffer.** 1980. Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate early protein VP175. J. Virol. **36:**189–203.
21. **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.
22. **Eisenberg, S. P., D. M. Coen, and S. L. McKnight.** 1985. Promoter domains required for expression of plasmid-borne copies of the herpes simplex virus thymidine kinase gene in virus-infected mouse fibroblasts and microinfected frog oocytes. Mol. Cell. Biol. **5:**1940–1947.
23. **Everett, R. D.** 1984. A detailed analysis of an HSV-1 early promoter: sequences involved in trans-activation by viral immediate-early gene products are not early gene specific. Nucleic Acids Res. **12:**3037–3056.
24. **Everett, R. D.** 1984. Transactivation of transcription by herpes virus product: requirement for two HSV-1 immediate early polypeptides for maximum activity. EMBO J. **3:**3135–3141.
25. **Faber, S. W., and K. W. Wilcox.** 1986. Association of the herpes simplex virus regulatory protein ICP4 with specific nucleotide sequences. Nucleic Acids. Res. **14:**6067–6083.
26. **Flores, O., L. Hua, and D. Reinberg.** 1992. Factors involved in specific transcription by mammalian RNA polymerase II. Identification and characterization of factor IIH. J. Biol. Chem. **267:**2786–2793.
27. **Flores, O., H. Lu, M. Killeen, J. Greenblatt, Z. F. Burton, and D. Reinberg.** 1991. The small subunit of transcription factor IIF recruits RNA polymerase II into the preinitiation complex. Proc. Natl. Acad. Sci. USA **88:**9999–10003.
28. **Gallinari, P., K. Wienbauer, M. C. Nardi, and J. Jiricny.** 1994. Localization of a 34-amino acid segment implicated in the dimerization of the herpes simplex virus type 1 ICP4 polypeptide by a dimerization trap. J. Virol. **68:**3809–3820.
29. **Ge, H., and R. G. Roeder.** 1994. Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. Cell **78:**513–523.
30. **Gelman, I. H., and S. Silverstein.** 1985. Identification of immediate early genes from herpes simplex virus that transactivate the virus thymidine kinase gene. Proc. Natl. Acad. Sci. USA **82:**5265–5269.
31. **Godowski, P. J., and Knipe, D. M.** 1986. Transcriptional control of herpes virus gene expression: gene functions required for positive and negative regulation. Proc. Nat. Acad. Sci. USA **83:**256–260.
32. **Gu, B., and N. Deluca.** 1994. Requirements for activation of the herpes simplex virus glycoprotein C promoter in vitro by the viral regulatory protein ICP4. J. Virol. **68:**7953–7965.
33. **Gu, B. and N. DeLuca.** Unpublished observations.
34. **Gu, B., R. Kuddus, and N. A. DeLuca.** 1995. Repression of activator-mediated transcription by herpes simplex virus via a mechanism involving interactions with the basal transcription factors TATA-binding protein and TFIIB. Mol. Cell. Biol. **15:**3618–3626.
35. **Gu, B., R. Rivera-Gonzalez, C. A. Smith, and N. A. DeLuca.** 1993. Herpes simplex virus infected cell polypeptide 4 preferentially represses Sp1-activated over basal transcription from its own promoter. Proc. Natl. Acad. Sci. USA **90:**9528–9532.
36. **Ha, I., W. S. Lane, and D. Reinberg.** 1991. Cloning of a human gene encoding the general transcription initiation factor IIB. Nature (London) **532:**689–695.
37. **Halpern, M. E., and J. R. Smiley.** 1984. Effects of deletions on expression of the herpes simplex virus thymidine kinase gene from the intact viral genome: the amino terminus of the enzyme is dispensable for catalytic activity. J. Virol. **50:**733–738.
38. **Hansen, S. K., and R. Tjian.** 1995. TAFs and TFIIA mediate differential utilization of the tandem *Adh* promoters. Cell **82:**565–575.
39. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpes virus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14:**8–19.
40. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpes virus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. USA **72:**1276–1280.
41. **Imbalzano, A. N., D. Coen, and N. A. DeLuca.** 1991. Herpes simplex transactivator ICP4 operationally substitutes for the cellular transcription factor Sp1 for efficient expression of the viral thymidine kinase gene. J. Virol. **65:**565–574.
42. **Imbalzano, A. N., and N. DeLuca.** 1992. Substitution of a TATA box from a herpes simplex virus late gene in the viral thymidine kinase promoter alters ICP4 inducibility but not temporal expression. J. Virol. **65:**5453–5463.
43. **Imbalzano, A. N., A. A. Shepard, and N. A. DeLuca.** 1990. Functional relevance of specific interactions between herpes simplex virus type 1 ICP4 and sequences from the promoter-regulatory domain of the viral thymidine kinase gene. J. Virol. **64:**2620–2631.
44. **Inostroza, J., O. Flores, and D. Reinberg.** 1991. Factors involved in specific transcription by mammalian RNA polymerase II: purification and functional

analysis of general transcription factor IIE. J. Biol. Chem. **266:**9304–9308.
45. **Kao, C., P. M. Lieberman, M. C. Schmidt, Q. Zhou, R. Pei, and A. J. Berk.** 1990. Cloning of a transcriptionally active human TATA binding factor. Science **248:**1646–1650.
46. **Kretzschmar, M., K. Kaiser, F. Lottspeich, and M. Meisterernst.** 1994. A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators. Cell **78:**525–534.
47. **Kuddus, R., B. Gu, and N. A. DeLuca.** 1995. Relationship between TATA-binding protein and herpes simplex virus type 1 ICP4 DNA-binding sites in complex formation and repression of transcription. J. Virol. **69:**5568–5575.
48. **Lieberman, P.** 1994. Identification of functional targets of Zta transcriptional activator by formation of stable preinitiation complex intermediates. Mol. Cell. Biol. **14:**8365–8375.
49. **Lin, Y. S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator in vitro. Cell **67:**365–376.
50. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. J. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex type 1. J. Gen. Virol. **69:**1531–1574.
51. **McGeoch, D. J., A. Dolan, S. Donald, and D. H. K. Brauer.** 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res. **14:**1727–1744.
52. **Meisterernst, M., A. L. Roy, H. M. Lieu, and R. G. Roeder.** 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. Cell **66:**981–993.
53. **Metzler, D. W., and K. W. Wilcox.** 1985. Isolation of herpes simplex virus regulatory protein ICP4 as a homodimeric complex. J. Virol. **55:**329–337.
54. **Michael, N., and B. Roizman.** 1993. Repression of the herpes simplex virus 1 a4 gene by its gene product occurs within the context of the viral genome and is associated with all three identified cognate sites. Proc. Natl. Acad. Sci. USA **90:**2286–2290.
55. **Nakajima, N., M. Horikoshi, and R. G. Roeder.** 1988. Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA promoter interactions of TFIID. Mol. Cell. Biol. **8:**4028–4040.
56. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular-weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53:**751–760.
57. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56:** 723–733.
58. **Paterson, T., and R. D. Everett.** 1988. Mutational dissection of the HSV-1 immediate-early protein Vmw175 involved in transcriptional transactivation and repression. Virology **166:**186–196.
59. **Paterson, T., and R. D. Everett.** 1990. The regions of the herpes simplex virus type 1 immediate early protein Vmw175 required for site specific DNA binding closely correspond to those involved in transcriptional regulation. Nucleic Acids Res. **16:**11005–11025.
60. **Pereira, L., M. H. Wolff, M. Fenwick, and B. Roizman.** 1977. Regulation of herpes virus macromolecular synthesis. V. Properties of a polypeptide made in HSV-1 and HSV-2 infected cells. Virology **77:**733–749.
61. **Post L. E., S. Mackem, and B. Roizman.** 1981. Regulation of alpha genes of HSV: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. Cell **24:**555–565.
62. **Preston, C. M.** 1979. Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant *tsK*. J. Virol. **29:**275–284.
63. **Quinlan, M., and D. Knipe.** 1985. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. Mol. Cell. Biol. **5:**957–963.
64. **Reinberg, D., and R. Roeder.** 1987. Factors involved in specific transcription by mammalian RNA polymerase II: purification and functional analysis of initiation factors IIB and IIE. J. Biol. Chem. **262:**3310–3321.
65. **Roberts, M. S., A. Boundy, P. O'Hare, M. C. Pizzorno, D. M. Ciufo, and G. S. Hayward.** 1988. Direct correlation between a negative autoregulatory response element at the cap site of the herpes simplex virus type 1IE175(a4) promoter and a specific binding site for the IE175(ICP4) protein. J. Virol. **62:**4307–4320.
66. **Ruppert, S., and R. Tjian.** 1995. Human $TAF_{II}250$ interacts with RAP74: implications for RNA polymerase II initiation. Genes Dev. **9:**2747–2755.
67. **Ruppert, S., E. H. Wang, and R. Tjian.** 1993. Cloning and expression of human $TAF_{II}250$: a TBP-associated factor implicated in cell-cycle regulation. Nature (London) **362:**175–179.
68. **Samaniego, L. A., A. L. Webb, and N. A. DeLuca.** 1995. Functional interactions between herpes simplex virus immediate-early proteins during infection: gene expression as a consequence of ICP27 and different domains of ICP4. J. Virol. **69:**5705–5715.
69. **Sawadogo, M., and R. G. Roeder.** 1985. Interaction of a gene specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell **43:**165–175.
70. **Shepard, A. A., and N. A. DeLuca.** 1989. Intragenic complementation among partial peptides of herpes simplex virus regulatory protein ICP4. J. Virol. **63:**1203–1211.
71. **Shepard, A. A., and N. A. DeLuca.** 1991. Activities of heterodimers composed of DNA-binding- and transactivation-deficient subunits of the herpes simplex virus regulatory protein ICP4. J. Virol. **65:**299–307.
72. **Shepard A. A., A. N. Imbalzano, and N. A. DeLuca.** 1989. Separation of primary structural components conferring autoregulation, transactivation, and DNA-binding properties to the herpes simplex virus transcriptional regulatory protein ICP4. J. Virol. **63:**3714–3728.
73. **Shepard, A. A., P. Tolentino, and N. A. DeLuca.** 1990. *trans*-dominant inhibition of the herpes simplex virus transcriptional regulatory protein ICP4 by heterocomplex formation. J. Virol. **64:**3916–3926.
74. **Smale, S. T., and D. Baltimore.** 1989. The "initiator" as a transcription control element. Cell **57:**103–113.
75. **Smiley, J. R., D. C. Johnson, L. I. Pizer, and R. D. Everett.** 1992. The ICP4 binding sites in the herpes simplex virus type 1 glycoprotein (gD) promoter are not essential for efficient gD transcription during virus infection. J. Virol. **66:**623–631.
76. **Smith, C. A., P. Bates, R. Rivera-Gonzalez, B. Gu, and N. DeLuca.** 1993. ICP4, the major regulatory protein of herpes simplex virus type 1, forms a tripartite complex with TATA-binding protein and TFIIB. J. Virol. **67:**4676–4687.
77. **Thut, C. J., J.-L. Chen, R. Klemm, and R. Tjian.** 1995. p53 transcriptional activation mediated by coactivators $TAF_{II}40$ and $TAF_{II}60$. Science **267:**100–104.
78. **Verrijzer, C. P., J.-L. Chen, K. Yokomori, and R. Tjian.** 1995. Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. Cell **81:**1115–1125.
79. **Verrijzer, C. P., K. Yokomori, J.-L. Chen, and R. Tjian.** 1994. *Drosophila* $TAF_{II}150$: similarity to yeast gene TSM-1 and specific binding to core promoter DNA. Science **264:**933–941.
80. **Watson, R., and J. B. Clements.** 1980. A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature (London) **285:**329–330.
81. **Xia, K., D. K. Knipe, and N. DeLuca.** 1996. Role of protein kinase A and the serine-rich region of herpes simplex virus type 1 ICP4 in viral replication. J. Virol. **70:**1050–1060.
82. **Zhou, Q., P. M. Leiberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964–1974.