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Mapping of the equine herpesvirus type 1 immediate-early protein interaction domain within the general transcription factor human TFIIB

Hyung-Kwan Jang[1], Jeong-Gon Cho, Hee-Jong Song

Department of Infectious Diseases, College of Veterinary Medicine, and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Korea
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Abstract

We previously reported that the equine herpesvirus type 1(EHV-1) immediate- early protein (IE protein) physically interacts with the general transcription factor human TFIIB(Jang et al, *J Virol* 75 : 10219-10230, 2001). The interaction between the IE protein and TFIIB is necessary for the IE protein to efficiently transactivate the early TK and late IR5 EHV-1 promoters. A panel of deletion and truncation mutants of the TFIIB gene was constructed and employed in protein-binding assays to map the IE protein-binding domain within TFIIB. Evidence is presented that the first direct repeat of TFIIB interacts specifically with the EHV-1 IE protein.

Key words : Equine herpesvirus type 1, EHV-1, IE protein, TFIIB

Introduction

Equine herpesvirus type 1(EHV-1) is a member of the subfamily *Alphaherpesvirinae*, and is employed as a model system to study multiple aspects of herpesvirus infections including gene function as well as regulation of viral gene expression. The EHV-1 genome contains 77 genes that are coordinately and temporally expressed as immediate-early, early, late gamma-1, and true late gamma-2 genes, as is the case for other herpesviruses[1~7]. The coordinated transcription of the viral genes is regulated by five regulatory proteins expressed as one immediate-early(IE) protein, three early proteins, and one late protein.

The IR1 gene maps within each inverted

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[1]Corresponding author
Phone : +82-63-270-3885, Fax : +82-63-270-3780
E-mail : hkjang@chonbuk.ac.kr

repeat region and encodes the 200-kDa IE phosphoprotein of 1,487 amino acids that is essential for EHV-1 replication[8~10]. The IE protein (i) represses transcription from its own promoter[11], (ii) potently activates expression of early promoters[11,12], and (iii) cooperates in a synergistic manner with the early EICP22 and EICP27 proteins to transactivate early and some late promoters[13~16]. A potent acidic transactivation domain within the first 89 amino acids of the IE protein is necessary for its activation properties[17]. In addition, the IE protein possesses a DNA-binding domain within amino acids 422 to 597 that specifically binds to the consensus DNA sequence 5'-ATCGT-3'[18]. Depending on the location of this consen- sus sequence relative to the transcription initiation start site, the IE protein either transactivates or represses transcription of certain genes[18,19]. An efficient nuclear localization signal was mapped to amino acids 963 to 970 of the IE protein that is necessary for the activation properties of the IE protein[12].

Interestingly, an early gene(IR2) maps within and is 3' coterminal with the IR1 gene[20]. The IR2 protein is unable to activate gene expression, which may be attributed to the absence of the acidic activation domain and serine-rich tract that are present with the first 322 residues of the native IE protein[17]. The function of the IR2 protein during EHV-1 infections remains an enigma, however the observa- tion that the IR2 protein retains most of the functional domains of the IE protein suggests that this protein functions as a negative regulator of some EHV-1 promoters, including the IE promoter.

General transcription factor TFIIB is a DNA-binding protein that functions in multiple stages of transcription[21,22]. TFIIB associates early with preinitiation complexes, influences transcription start site selection, stabilizes the interaction of TATA-binding protein and the TATA element, and recruits the RNA polymerase II-TFIIF complex[23~32]. TFIIB recruitment to promoters proceeds the initial binding of the TFIID-TFIIA(D-A) complex to promoters[33]. Because TFIIB influences several aspects of transcription initiation, various viral transactivators activate viral gene expression by recruiting TFIIB[34~40].

Recruitment of TFIIB into assembling preinitiation complexes on EHV-1 promoters may be a rate-limiting step that is enhanced by the IE protein. Interactions of the IE protein with TFIIB may induce conformational changes in TFIIB that increase (i) its association with TFIID, (ii) the stability of the TFIID-TFIIA-DNA complex, and/or (iii) the recruitment of the holoenzyme complex.

In this report, we further present our findings that the EHV-1 IE protein can physically interact and functionally cooperate with one of the general transcription factors, TFIIB, to stimulate transcription synergistically as our previous report[41]. Using a variety of protein-binding assays, we have precisely mapped the binding domain that mediate physical interaction of the EHV-1 IE protein within the general transcription factor human TFIIB.

Materials and Methods

Cell culture, virus infection, and preparation of nuclear extracts from infected cells

Cultures of murine fibroblast L-M cells were grown as monolayers in Eagle's minimum essential medium(EMEM) supplemented with penicillin(100 μg/mℓ), strepto-

mycin(100 μg/mℓ), nonessential amino acids, and 5% fetal bovine serum(FBS)[1,11]. Nuclear extracts of infected cells were prepared as described previously, with some modifications[42]. L-M cells(2.3×10^7) were infected with wild-type EHV-1 Kentucky A(KyA) strain at a multiplicity of infection (MOI) of 15 to 20 PFU per cell. At 6 hours post infection, cells were scraped into phosphate-buffered saline(PBS) containing 0.1 mM each of TLCK and TPCK, pelleted, and resuspended in 4 volumes of buffer A (10 mM HEPES at pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5% NP40, 0.5 mM DTT, 0.1 mM TLCK, and 0.1 mM TPCK). After incubation for 10 min on ice, the nuclei were pelleted at 14,000 rpm for 5 min in a microcentrifuge. The supernatant was discarded, and proteins were eluted from the nuclei by incubation for 30 min on ice in 2 volumes of buffer B(10 mM HEPES at pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 10 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT, 0.1 mM TLCK, and 0.1 mM TPCK). The nuclear debris were pelleted by centrifugation at 14,000 rpm for 15 min in a microcentrifuge, and the supernatants containing the nuclear IE proteins were stored at -70℃.

Plasmid constructions

All recombinant DNA methods were performed according to standard protocols[43]. Two expression plasmids(pN254[44] and pM270) carrying the entire human TFIIB gene were kindly provided by Dr D Reinberg (Massachusetts Institute of Technology, Cambridge, MA) and Dr M Hampsey(Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, NJ).

(1) GST-TFIIB fusion plasmids

To facilitate the mapping of the EHV-1 IE protein interaction domain within TFIIB, a panel of glutathione *S*-transferase(GST) hIIB deletion mutants was constructed(Fig 1A). Plasmid GSTKG- hIIB, which contains the entire TFIIB coding sequence cloned in frame with the GST gene and expresses the fusion protein GST-hIIB(1-316), was described previously[41]. Digesting pGBKhIIB with *Sma*I and *Sal*I, and ligating the released TFIIB gene to pGEX-4T1(Promega) that was digested with *Sma*I and *Sal*I generated plasmid GST4T1-hIIB. Plasmid GST-hIIBΔ4-37 was generated by a two step cloning strategy. First, pGSTKG- hIIB was digested with *Acc*I and *Sal*I, followed with blunt ending the 5′ end with Klenow enzyme, and inserting a *Cla*I linker d (pCCCATCGATGGG) (New England Biolabs) at codon 4 to generate pGSTKG- hIIBaa4C. Plasmid GSTKG- hIIBaa4C was subsequently digested with *Cla*I and *Hind*III and ligated with a PCR- amplified segment of TFIIB spanning codons 38 to 311. The forward primer was 5′-CCATCGATGGC-TTGGTTGTAGGTGACCGGG-3′ and the reverse primer was 5′-CCCAAGCTTTT- A TAGCTGTGGTAGTTTGTC-3′. GST- hIIB Δ4-66 is a derivative of pGSTKG- hIIBaa4C. Plasmid GSTKG-hIIBaa4C was digested with *Cla*I and *Hind*III and ligated with a PCR-amplified segment of TFIIB spanning codons 67 to 311. The forward primer was 5′-CCATCGATGTTGGAGA-TTCTCAGAA TCCTC-3′ and the reverse primer was 5′-CCCAAGCTTTTATAGC-TGTGGTAGT TTGTC-3′. Plasmid GST- hIIBΔ4-123 is a subclone of pGSTKG- hIIBaa4C, and was generated by inserting a PCR-amplified segment of TFIIB corresponding to codons 124

to 311. The forward primer was 5′-CCATCGATATG-GCAGACAGAATCAATC TAC-3′ and the reverse primer was 5′-CCCAAGCTTTT-ATAGCTGTGGTAGTTT GTC-3′. Plasmid GST-hIIBΔ67-123 was derived from pGST4T1-hIIBaa67N, which was generated by partially digesting pGST-4T1-hIIB with *Xho*I, blunt ending the 5′ end, and inserting the *Nco*I linker d(pCC-CATGGG) (New England Biolabs) at codon 67. Plasmid GST4T1-hIIBaa67N was subsequently digested with *Nco*I and religated to generate pGST-hIIBΔ67-123. Plasmid GST-hIIBΔ125-174 was derived from pGSTKG-hIIBaa174N, which was generated by digesting pGSTKG-hIIB with *Avr*II, blunt ending the 5′ end and inserting the *Nco*I linker d(pCAGCCATG- GCTG) (New England Biolabs).

Plasmid GSTKG-hIIBaa174N was subsequently digested with *Nco*I and religated to generate pGST-hIIBΔ125-174. Plasmid GST-hIIBΔ176-201 was generated by digesting pGSTKG-hIIB with *Avr*II and *Eco47*III, blunt ending the 5′ end with Klenow enzyme, and religating the resulting DNA fragment. Plasmid GST- hIIBΔ202-269 was cloned by digesting pGSTKG-hIIB with *Eco47*III and *Ppu*MI, blunt-ending the 5′ end, and religated. Plasmid GST-hIIBΔ271-297 was derived from pGSTKG- hIIBaa270B, which was cloned by digesting pGSTKG-hIIB with *Ppu*MI, blunt ending the 5′ end, and inserting the *Bgl*II linker d(pGGAAGAT-CTTCC) (New England Biolabs).

Plasmid GSTKG-hIIBaa270B was subsequently digested with *Bgl*II and religated to generate pGST-hIIBΔ271-297. Plasmid GST-hIIBΔ201-316 was cloned by digesting pGSTKG-hIIB with *Eco47*III and *Hind*III, blunt ending the 5′ end and religating the resulting DNA fragment. Plasmid GST-hIIBΔ271-316 was cloned by digesting pGSTKG-hIIB with *Ppu*MI and *Hind*III, blunt ending the 5′ end, and religating the resulting DNA fragment. pGST-hIIB(1-123) was created by digesting pGSTKG-hIIB with *Nco*I and *Hind*III, filling in the 5′ overhang with Klenow enzyme, and self-ligating the DNA fragment. pGST-hIIB(67-200) was cloned by digesting pGST-hIIBΔ4-66 with *Eco47*III and *Hind*III and self-ligating the resulting Klenow-treated restriction enzyme fragment. pGST-hIIB(175-316) was generated by cutting pGST-hIIBΔ4-66 with *Cla*I and *Avr*II and self-ligating the resulting Klenow-treated restriction enzyme fragment.

(2) IE and TFIIB mutant plasmids for *in vitro* transcription and translation

Plasmids pGST-IE and pGST-IE(407-757), which express IE amino acids 1 to 1487 and 407 to 757 as GST fusion proteins, respectively, were discussed previously[18,41]. G3IE and pGEM44, which express IE(1-1,487) and IE (323-1487; IR2 protein), respectively, from the Sp6 promoter in *in vitro* transcription and translation reactions employing the T$_N$T coupled rabbit reticulocyte lysate system(Promega) were described previously[20,41]. Plasmid pG3hIIB, which expresses the entire TFIIB gene in *in vitro* transcription and translation reactions was described earlier[41]. Plasmid G3hIIBΔ125-174 was used expressed in *in vitro* transcription and translation reactions to generate TFIIBΔ125-174. Plasmid G3h-IIBΔ125-174 was generated by cloning the *Bst*EII-*Bgl*II fragment of pGST-hIIBΔ125-174 into the *Bst*EII-*Bgl*II sites of pG3hIIB(1-316).

Purification of GST fusion proteins

Expression and purification of GST fusion proteins were carried out by a modification of the purification procedures described elsewhere[14,18,45]. The pGEX expression vectors encoding IE or each TFIIB derivative were transformed into the *E coli* BL21(DE3) pLysE strain. The transformed bacteria were grown overnight at 37℃ in 2X YT(yeast extract and tryptone) medium supplemented with 2% glucose and the appropriate antibiotics(100 ㎍/㎖ of ampicillin and 34 ㎍/㎖ of chloramphenicol). The cultures were diluted 1 : 100 in 250 ㎖ of fresh, pre-warmed 2× YT medium containing the appropriate antibiotics and grown for 1.5 to 2 hours at 37℃. Fusion protein synthesis was then induced by incubating the cells with 0.5 mM isopropyl-D- thiogalactoside(IPTG) for 2 to 3 hours at 37℃. The cells were lysed, and the proteins were purified with the BugBuster GST-Bind Purification Kit (Novagen) according to the manufacturer's instructions with slight modifications. Cells were lysed in 12 ㎖ of protein extraction reagent containing 25 U/㎖ of Benzonase nuclease for 20 min with shaking at room temperature(RT). Insoluble debris were removed by centrifugation at 12,000 rpm for 20 min in the Beckman JA-20 rotor, and GST proteins were purified from the soluble extract by batch-binding the supernatant with GST-bind resin(1 ㎖ of resin per 5~8 ㎎ of protein) at RT for 40 min. The beads were then washed twice with 5 ㎖ of GST bind/wash buffer. The bound proteins were eluted from the resin in 1.5 to 2 ㎖ of GST elute buffer. Eluates were then loaded into Centricon columns(Amicon) as directed by the manufacturer to both desalt and concentrate the purified proteins by ultrafiltration. Protein purity was determined by SDS-PAGE, and concentrations were estimated by densitometric analysis using the Gel Doc 1000/2000 gel documentation system(Bio-Rad) by comparing protein intensity to known amounts of bovine serum albumin (Pierce). Aliquots of proteins were stored at -70℃.

In vitro transcription and translation

All of the *in vitro* expression plasmids used in this study were transcribed with Sp6 RNA polymerase and translated in rabbit reticulocyte lysate in the presence of [^{35}S]methionine(40 Ci/㎖; specific activity, 1,175 Ci/mmol; New England Nuclear Corp) as recommended by the manufacturer (Promega). Experiments involving the *in vitro*-synthesized proteins were performed in the presence of ^{35}S- labeled proteins. Radioactive products were analyzed by SDS-PAGE followed by autoradiography, and either stored at -70℃ or used immediately for *in vitro* protein- binding assays.

Protein-binding assay

Protein-protein interactions of the EHV-1 IE proteins(*in vitro*-synthesized, bacterial expressed, or nuclear extracted) with TFIIB were carried out by a modification of described procedures[45]. Aliquots of proteins were thawed on ice, and 2 ㎍ of the appropriate GST fusion protein was incubated with the ^{35}S-labeled proteins in a final volume of 600 ㎕ of NETN buffer(100 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl at pH 8.0, 0.5% NP40). After incubation for 90 min at RT with gentlerocking, 30 ㎕ of a 50% slurry of gluta-thione-Sepharose beads (Pharmacia) was added, and the proteins were incubated an additional hour at RT.

The beads were then centrifuged and washed five times with 600 ㎕ of NETN buffer. The bound proteins were eluted by boiling for 5 min in 20 ㎕ of 2X SDS sample buffer(120 mM Tris-HCl at pH 6.8, 4% SDS, 20% glycerol, 0.001% bromphenol blue, 2% 2-mercaptoethanol), and analyzed by SDS-PAGE. The gels were dried, and the bands were quantitated by PhosphorImager analysis (Molecular Dynamics).

Western blot analysis and antibody

Proteins were separated in 8% SDS-PAGE gels and electro-transferred to a nitrocellulose membrane(Bio-Rad) for 1 hour. After transfer, the membrane was blocked for 1 hour at RT in TBST buffer(10 mM Tris-HCl at pH 8.0, 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat powdered milk. The membrane was then incubated with anti-IE peptide-specific antiserum at a dilution of 1 : 1,000 in TBST for 30 min at RT. After three 10 min washes with TBST, the membrane was incubated with alkaline phosphatase-conjugated goat anti-rabbit antibody(Sigma), diluted in TBST at a dilution of 1 : 5,000, for 30 min at RT, and followed with three TBST washes to remove unbound antibody. Immunocomplexes were visualized by incubation in AP buffer(100 mM Tris-HCl at pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) containing the AP substrates BCIP/NBT(0.165 ㎎/㎖ of 5-bromo-4-chloro-3-indo-lylphosphate p-toluidine salt [BCIP] and 0.3 ㎎/㎖ of nitroblue tetrazolium chloride [NBP]; Gibco BRL). The anti-IE peptide-specific antiserum was raised against a peptide derived from amino acids 925 to 943 of the IE protein and has been demonstrated in previous studies to be highly reactive to the IE protein[9,17].

Results

Mapping the domain of the TFIIB protein required for the IE protein-TFIIB interaction

To map the IE protein-binding domain within TFIIB, the purified GST-hIIB fusion proteins(Figs 1A and 1B) were employed in

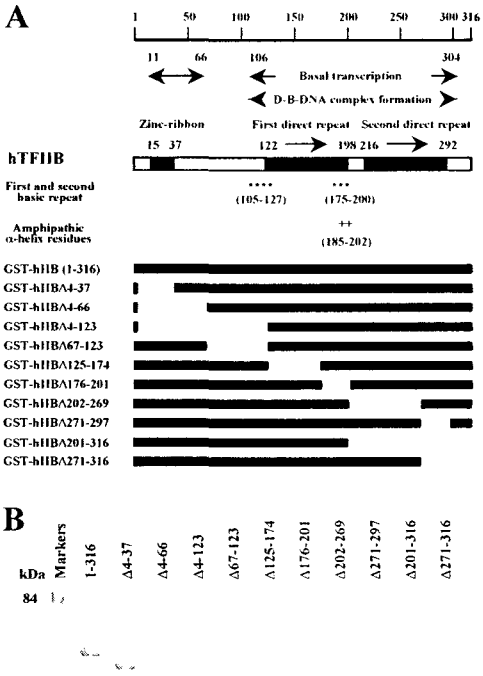

Fig 1. Deletion mutants of the GST-hIIB fusion protein employed to map the IE protein-binding domain within TFIIB.
Panel A: Schematic representation of GST-hIIB fusion proteins tested in protein-binding assays. The amino acid positions of the TFIIB functional domains are indicated at the top of the figure. The amino acid sequence that is deleted in each TFIIB mutant is indicated to the left. Panel B: Coomassie blue-stained polyacrylamide gel of the *E coli* expressed GST-hIIB fusion proteins. kDa molecular weight markers are indicated on the left.

GST-pulldown assays as described in materials and methods. The results of the initial GST-pulldown assays involving [^{35}S]methionine-labeled IE or IR2 protein are presented in Figs 2A and 2B, respectively. As expected from our previous studies, GST-hIIB(1-316) interacted with the *in vitro* transcription and translation-generated IE and IR2 proteins. The deletion mutants GST-hIIBΔ4-37, GST-hIIBΔ4-66, GST-hIIBΔ4-123, GST-hIIBΔ67-123, GST-hIIBΔ176-201, GST-hIIBΔ202-269, GST-hIIBΔ271-297, GST-hIIBΔ201-316, and GST-hIIBΔ271-316 precipitated the IE and IR2 proteins with relatively the same efficiency as the full-length TFIIB protein. However, GST-hIIBΔ125-174 was not able to precipitate either of the *in vitro* transcription and translation-synthesized EHV-1 proteins (Figs 2A and 2B) or from infected cell nuclear extracts (Fig 3), which indicates that amino acids 125 to 174 within the first direct repeat of TFIIB contains an IE protein-binding domain.

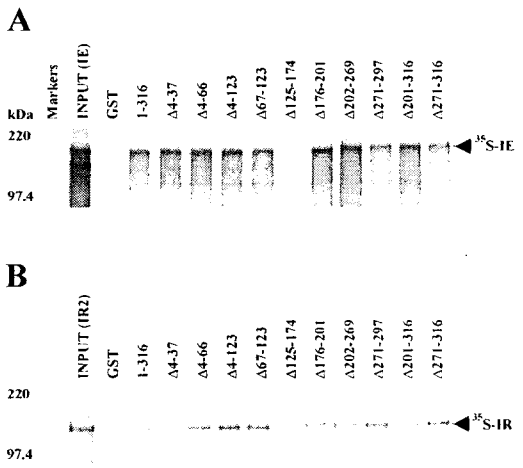

Fig 2. Mapping the IE protein-binding domain within TFIIB.
Panel A: The GST-hIIB proteins were assayed in GST-pulldown assays for inter-action with ^{35}S-IE protein. Panel B: Parallel GST-pulldown assays tested the ability of the GST-hIIB proteins to precipitate ^{35}S-IR2 protein. In panels A and B, input lanes represent the amount of IE or IR2 proteins included in each reaction to assess the relative binding efficiency of each GST-hIIB fusion protein.

Confirming the IE protein interaction domain within TFIIB using native IE protein

To confirm the above mapping results, the ability of the panel of GST-hIIB fusion proteins to react with native IE protein was examined.

The binding reactions were repeated as described above with 20 μl of nuclear extracts prepared from L-M cells mock-infected or infected for 6 hours with EHV-1 KyA at an MOI of 15 to 20. After washing the resin, the precipitated protein complexes were resolved by SDS-PAGE analysis. The presence of the native IE protein within each precipitate was indicated by Western blot analysis with an anti-IE protein(amino acids 925 to 943) polyclonal antibody[14]. The results of the Western blot analyses of the cell nuclear extract precipitates are presented in Fig 3. As was the case for the *in vitro* transcription and translation generated proteins, GST-hIIB(1-316) interacted with the native IE protein from infected cell nuclear extracts, while GST-hIIBΔ125-174 was defective in precipitating the native protein. These results indicate that amino acids 125 to 174 within the first direct repeat of TFIIB contain an IE protein-binding domain. The inability of GST alone to precipitate the test proteins indicates the specificity of the GST-pulldown assays.

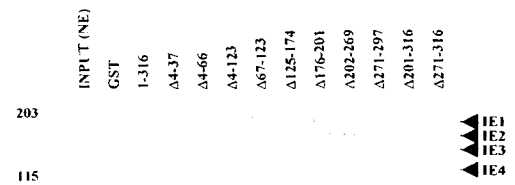

Fig 3. Interaction of the full-length and mutant hIIB with the native IE protein from nuclear extracts of EHV-1 KyA-infected L-M cells.
IE1 to IE4 represent a family of IE species that were characterized elsewhere[8]. The input lane represents the amount of native IE proteins included in each reaction to assess the relative binding efficiency of each GST-hIIB fusion protein. Whereas native GST-hIIB interacted efficiently with IE protein and IR2 protein, deletion of amino acids 125 to 174 within the first direct repeat of TFIIB abolished its interaction with the EHV-1 proteins.

TFIIB protein amino acids 125 to 174 specifically interact with the IE protein

To confirm that amino acids 125 to 174 of TFIIB specifically interact with the IE and IR2 proteins, the ability of different forms of IE protein produced as GST fusion proteins to precipitate [^{35}S]methionine-labeled TFIIB or TFIIB△125-174 was examined(Fig 4). We previously reported amino acids 407 to 757 of the IE protein harbors a bipartite TFIIB-binding domain[41]. Therefore, GST-IE(407-757) was included in these studies to further exemplify the specific interaction between the IE protein and TFIIB. As shown in Fig 4, 2 ㎍ of GST-IE protein(1-1487), GST-IR2 protein(323-1487), and GST-IE(407-757) were able to precipitate full-length ^{35}S-labeled TFIIB with equal efficiency. These results reproduced our previous findings that the IE protein amino acid domain 407-757 contains a TFIIB-binding domain. Deletion of amino acids 125 to 174 of TFIIB inhibited the ability of the three GST fusion proteins to precipitate ^{35}S-TFIIB △125-174. These results streng-then the previous observations that the IE protein-binding domain maps to amino acids 125 to 174 within the first direct repeat of TFIIB.

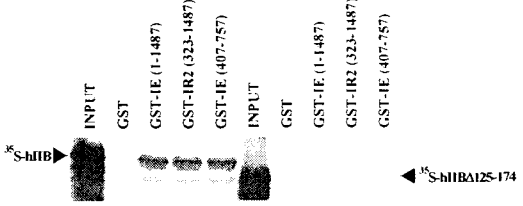

Fig 4. TFIIB amino acids 125 to 174 physical interact with the IE protein.
Each GST-IE fusion protein tested interacted efficiently with [^{35}S]methionine-labeled hTFIIB(1-316). However, deletion of the IE protein-binding domain within amino acids 125 to 174 abrogated this interaction with the GST-IE proteins. Input lanes represent the amount of ^{35}S-TFIIB or ^{35}S-TFIIB△125-174 included in each reaction and were employed to assess the relative binding efficiency of each GST fusion protein. The additional bands detected in the 35S-TFIIB precipitates most likely represents degradation of the in vitro transcription and translation product or minor protein species originating from internal translation start sites.

TFIIB protein amino acids 125 to 174 harbor an IE protein-binding domain

To further corroborate the mapping of an IE protein-binding domain, additional GST-

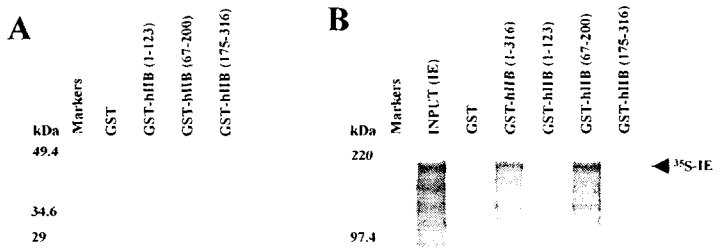

Fig 5. Amino acids 67 to 200 of TFIIB harbor the minimal IE protein-binding domain.
Panel A: Coomassie blue-stained polyacrylamide gel of the *E. coli* expressed GST-hIIB fusion proteins. Panel B: GST-pulldown assay assessed the ability of the GST fusion proteins to interact with the ^{35}S-IE protein. In both panels, kDa molecular weight markers are indicated on the left. The input lane represents the amount of ^{35}S-IE included in each reaction and was employed to assess the relative binding efficiency of each GST fusion protein. These results indicate that TFIIB amino acids 125 to 174 contain an IE protein-binding domain.

hIIB mutants(Fig 5A) were tested for interaction with ^{35}S-IE. In this experiment, the GST-hIIB proteins that contained the IE protein-binding domain within amino acids 125 to 174 interacted efficiently with ^{35}S-IE (Fig 5B). However, GST-hIIB(1-123) and GST-hIIB(175-316), which lack residues 125 to 174, were incapable of precipitating the IE protein. Thus, this assay demonstrated that the IE protein-binding domain resides within amino acids 125 to 174 of TFIIB.

Discussion

A common theme of viral regulatory proteins in activating gene expression is to interact with general transcription factors in a manner that enhances recruitment of the RNA polymerase II. Examples include the bovine papillomavirus E2 protein interactions with TFIIB and TBP[37,40], HSV-1 VP16 associations with TFIIB, TFIIA, and TAF$_{II}$40[46-50], HSV-1 ICP4 recruitment of TFIIB, TBP, and TAF$_{II}$250[52-54], and Epstein-Barr virus EBNA2 binding to TFIIB, TAF$_{II}$40, and RPA70[55]. In our accompanying paper, we presented evidence that IE residues 407 to 757 within the IE protein's helix-loop-helix harbor a TFIIB-binding domain[41]. In this paper, we mapped an IE protein-binding domain within TFIIB amino acids 125 to 174.

Previously, we suggested that upon dimerization of the IE protein, possibly via the helix-loop-helix, key hydrophobic residues within the TFIIB-binding domain become exposed and enable the IE protein to interact with TFIIB. TFIIB residues 125 to 174 that associate with the IE protein are located within the core domain of TFIIB. Analysis of the TFIIB amino acid sequence as well as the solution structure of the core domain revealed that this region of TFIIB contains a hydrophobic sequence(residues 159 to 168)[56]. The mapping of the IE protein-binding domain within the core region that contains this hydrophobic sequence supports our suggested mechanism by which the IE protein interacts with both DNA and TFIIB[41]. The ability of the IR2 protein to

bind with comparable efficiency as the IE protein to the identified domain within TFIIB, raises an interesting possibility. Since the IR2 protein lacks the potent acidic activation domain within amino acids 3 to 89 of the full-length protein, the IR2 protein may function as a negative regulator of viral gene expression by squelching the limited supply of transcription factors such as TFIIB. Based on the current data presented and findings discussed in our previous paper, we conclude that the interaction of the IE protein's TFIIB-binding domain(amino acids 407 to 757) with TFIIB amino acids 125 to 174 is necessary for the transactivation properties of the IE protein.

Based on the data presented and our finding that the IE protein forms dimers[44], we present the following model to explain how the IE protein possibly activates transcription of viral genes via recruitment of TFIIB. Initially, the IE protein homodimerizes via the adjacent alpha helices present within the DNA-binding helix-loop-helix domain(amino acids 422 to 597)[32]. This self-interaction results in a conformational change of the loop structure such that the loop becomes accessible to bind to the major groove at the consensus target sequence, ATCGT[18].

Concomitantly, dimerization of the IE protein may increase the exposure of key hydrophobic residues within the TFIIB-binding domain(amino acids 407 to 757), resulting in the increased efficiency with which the dimeric IE proteins bind to TFIIB. Computer analysis of TFIIB with the program ProtScale(http://www.expasy.ch/cgi-bin/protscale.pl?1) identifies regions of TFIIB that are rich in hydrophobic residues (i.e. amino acids 1 to 26, 55 to 64, 171 to 180, and 277 to 282), that could interact with the exposed hydrophobic residues within the IE protein's TFIIB-binding domain.

The findings in this report add the TFIIB to a growing list of proteins that interacts with the sole multi-functional IE protein of this alphaherpesvirus. Our recent studies have revealed that the IE protein interacts with itself[45], the EICP22 protein[45], cellular protein EAP[57], possibly with the EICP27 protein as well as proteins that post-transcriptionally modify the IE phosphoprotein and allow its transport to the nucleus(unpublished data). A major goal of our future endeavors will concern efforts to identify other viral proteins and cellular factors that interact with the IE protein and hopefully to gain some understanding as to how these interaction influence specific functions of this interesting and essential viral protein.

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