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Transcriptional Activation Domain of Human BTEB2, A GC Box-Binding Factor[1]

Satoshi Kojima,* Akira Kobayashi,* Osamu Gotoh,† Yoshiaki Ohkuma,‡ Yoshiaki Fujii-Kuriyama,* and Kazuhiro Sogawa*,[2]

*Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-77; †Department of Biochemistry, Saitama Cancer Center Research Institute, Inamachi, Saitama 362; and ‡Institute for Molecular and Cellular Biology, Osaka University, Yamada-oka, Suita, Osaka 565

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BTEB2 is a GC box-binding transcription factor containing proline-rich and zinc finger domains as transactivation and DNA binding domains, respectively. We have identified a small region in the proline-rich domain indispensable for its transcription-enhancing activity by transfection experiments using various expression plasmids with point mutations or small deletions in the domain. This region comprising about 10 amino acids was relatively hydrophobic and rich in proline and alanine residues. BTEB2 purified from a baculovirus expression system could enhance transcription, depending on the presence of GC boxes in the promoter region of templates in an *in vitro* transcription assay. BTEB2 deleted of the hydrophobic region, however, lost the transcription-enhancing activity, in confirmation of the above results. Basic transcription factors which possibly interact with BTEB2 were examined. Initiation factors, TFIIB, TFIIEβ, and TFIIFβ as well as the TATA box-binding protein (TBP) were found to interact with BTEB2 when analyzed by *in vitro* binding experiments, although these interactions could not be attributed to the proline-rich domain. We discussed factors which interact with and transmit the transcriptional activity of the BTEB2 activation domain to basic transcriptional machinery.

Key words: hydrophobic sequence, proline-rich domain, protein-protein interaction, transcription factor, zinc finger protein.

A GC box sequence is one of the most widely used *cis*-acting elements in the promoter region of eukaryotic genes including viral genes, house-keeping genes, and oncogenes (*1, 2*). As a factor binding to the GC box, Sp1 was first found and was shown to be involved in the transcription of the genes (*3-5*). Recently several proteins, BTEB (*6*), BTEB2 (*7*), EKLF (*8*), LKLF (*9*), Sp3 (*10, 11*), and Sp4 (*10, 11*), (Sp3 and Sp4 are also referred to as SPR-2 and SPR-1, respectively) with relatively small size were cloned and characterized as GC box-binding factors. These proteins and Sp1 commonly contain three contiguous Cys_2-His_2 zinc finger motifs at their C-terminal region as a DNA binding domain. The zinc finger domains of these proteins exhibit approximately 60 to 80% sequence similarity among them (*9*), and their modes of binding to the GC box are indistinguishable from one another (*7, 12*), while other regions which may possess transactivation activity and other transcriptional functions display no marked sequence similarity except for the similarity within a group of Sp1, Sp3, and Sp4 (*10, 11*).

A number of transcriptional activation domains of various transcription factors have been analyzed mainly by fusing their various parts with heterologous DNA binding domains such as the GAL4 DNA binding domain to assay transcription of the corresponding reporter genes cotransfected into cultured cells. Through these studies it has been established that the transcriptional activation domains are categorized by their characteristic amino acid sequence in the domain into glutamine-rich, proline-rich, and acidic activation domains (*4, 13, 14*). Other activation domains without apparently notable feature also exist. Transactivation domains of GC box-binding factors were not fully investigated, although Sp1 was reported to consist of two repeats of glutamine-rich sequences, representing a typical transcriptional activation (*4*). Another GC box-binding factor, BTEB revealed that its activation domain is not a typical one, but consists of two short hydrophobic stretches (*15*).

BTEB2, EKLF, and LKLF share a common feature with a proline-rich domain in the N-terminal half of the molecule, which is thought to serve as a transcriptional activation domain (*7-9*), although the sequences are totally different from one another. In addition, these three GC box-binding factors were expressed in the restricted tissues or cells (*7-9*), while Sp1 and BTEB were expressed ubiquitously (*4, 6, 16*). These features suggest that they play a key role different from Sp1 in transcriptional control of some tissue-specific genes. Indeed, it was clearly shown that EKLF was a factor necessary for β-globin synthesis in

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[2] To whom correspondence should be addressed. Tel: +81-22-217-6591, Fax: +81-22-217-6594

Abbreviations: TBP, TATA box-binding protein; BTE, basic transcription element; CAT, chloramphenicol acetyltransferase.

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developing embryos using EKLF-null mice produced by the targeted gene disruption method (*17, 18*). Accordingly, it is important to investigate how only a limited number of genes such as β-globin gene in thousands of genes containing GC boxes in their promoter region are selectively activated, and whether or not there is a common mechanism for the transcriptional activation among these three factors. As a first step toward understanding of the regulatory mechanism of these GC box-binding factors, we investigated the activation domain of BTEB2 in *in vivo* and *in vitro* transcription systems.

Introduction of point mutations or short deletions into the proline-rich domain of BTEB2 revealed a short hydrophobic segment necessary for the transcriptional activation of reporter genes in the transfection experiments. Furthermore, we describe an *in vitro* transcription assay system in which the transcription activity depends on the purified BTEB2 protein to confirm the importance of the hydrophobic region for the transcriptional activity.

MATERIALS AND METHODS

Plasmid Construction—pGALBTEB2 and pGALΔBTEB2 were constructed by inserting the 462 bp *Xba*I/*Dra*I fragment and the 222 bp *Pst*I/*Hpa*I fragment of human BTEB2 cDNA (*7*), respectively, into the *Bam*HI site of pSG424 (*19*).

PCR was used to generate point mutations in the proline rich region using pGALΔBTEB2 as a template as follows. The PCR was performed under the standard condition except that the concentrations of 4 dNTPs were elevated to 1 mM to increase the mutation rate, by 35 cycles, each of which cycle consisted of heating at 94°C for 1 min, at 55°C for 1 min, at 72°C for 1 min using a pair of synthetic primers, 5′-gtgaaacaattccagggcat-3′ and 5′-aactggcagggtgggtgggta-3′. A fraction (1/1,000) of the PCR products was used as a template for next amplification. After 8 cycles of amplification, the PCR products were cloned into the *Sma*I site of M13mp18 and sequenced. The plasmids were digested with a combination of *Bam*HI and *Kpn*I or *Kpn*I and *Xba*I according to their reading frame and the resultant fragments were cloned into the *Bam*HI/*Kpn*I or *Kpn*I/*Xba*I site of pSG424. In the case of the latter cloning site, pSG424 had been digested with *Sal*I, treated with Klenow fragment, and religated in order to make the inserted sequence in-frame.

To construct Δ89-96 we generated two fragments by the PCR method using two pairs of primers, 5′-tggaggtggggttaacttctggag-3′ and 5′-aagtgcgagatcatcatcgg-3′, and 5′-aaactggcaatgcataatccaaat-3′ and 5′-caggaaacagctatgac-3′, and M13GALBTEB2 (which was generated by inserting the *Hin*dIII/*Xba*I fragment of pGALBTEB2 into the *Hin*dIII/*Xba*I site of M13mp18) as a template. After digestion of the 5′ ends of the two fragments with *Bam*HI and *Xba*I, respectively, they were inserted into the *Bam*HI/*Xba*I site of pSG424. Δ86-100 was similarly constructed by using the same PCR fragments. After the 5′ fragment was digested with *Bam*HI/*Hpa*I and the 3′ fragment with *Xba*I/*Eco*T22I, they were inserted into the *Bam*HI/*Xba*I site of pSG424.

The 88P→S, 93T→I, 95A→V, 96S→P, and 98L→P were generated by site-directed mutagenesis using the following five oligonucleotides as primers on the template of pGALBTEB2, 5′-aacccaccttcatcctatg-3′ for 88P→S, 5′-atgctgctataattgcttct-3′ for 93T→I, 5′-ctacaattgtttctaaactg-3′ for 95A→V, 5′-acaattgctcctaaactggc-3′ for 96S→P, and 5′-cttctaaaccggcaattcac-3′ for 98L→P (the mutated nucleotides were underlined).

For expression in the baculovirus system, we constructed plasmids of BTEB2 and its mutant protein (BTEB2ΔAD) lacking the activation domain as follows. For construction of the expression plasmid for BTEB2, blunt-ended *Xba*I fragment from pCMBTEB2 and synthetic oligonucleotides (*20*) encoding six histidines were inserted into the *Bam*HI/*Sma*I site of pVL1393 (*21*). To construct BTEB2ΔAD expression plasmid, we first made an intermediate plasmid, which was constructed by inserting the 261 bp *Bam*HI/*Hpa*I fragment from Δ86-100 and the 395 bp *Hpa*I/*Xba*I fragment from pCMBTEB2 into the *Bam*HI/*Xba*I site of pBluescript II SK(+) (Stratagene). The *Sma*I fragment from the intermediate plasmid and the synthetic oligonucleotide (*20*) for his-tag were inserted into the *Xba*I site and the *Bam*HI/*Sma*I site, respectively, of pVL1393.

Templates (44, 53, and V) containing G-less cassette used for *in vitro* transcription experiments were described previously (*22*).

To produce plasmids for synthesis of BTEB2 and BTEB2AD proteins in the *in vitro* translation system, the 775 bp *Xba*I fragment from pCMBTEB2 and the 492 bp *Bam*HI/*Xba*I fragment from pGALBTEB2 were inserted into the *Xba*I site and the *Bam*HI/*Xba*I site, respectively, of pBluescript II SK(+) to be able to transcribe for *in vitro* transcription under the direction of the T7 promoter. The expression plasmid for BTEB2ZF was made by excising the *Nco*I/*Hpa*I fragment from the expression plasmid for BTEB2, followed by the treatment with Klenow fragment and subsequently by self-ligation.

DNA Transfection and CAT Assay—DNA transfection was performed by the calcium phosphate-DNA coprecipitation method as described (*23*). CAT assays were carried out with different DNA preparations by the method of Gorman *et al.* (*24*).

Baculovirus Expression System—Wild type baculovirus genomic DNA was cotransfected with the expression plasmids for BTEB2 and BTEB2ΔAD into Sf9 cells as described (*20, 21*). The recombinant baculovirus clone was selected by morphological screening of plaques. BTEB2 and BTEB2ΔAD were purified from crude extracts of virus-infected Sf9 cells by the nickel affinity chromatography as described (*20*).

In Vitro Transcription Assay—Nuclear extracts were prepared from Sf9 cells (*21*) as described by Dignam *et al.* (*25*). Reactions were performed in 30 μl of 13.3 mM HEPES (pH 7.9), 67 mM KCl, 13.3% (v/v) glycerol, 0.13 mM EDTA, 0.33 mM PMSF, 0.67 mM DTT, 6 mM $MgCl_2$, 4 U RNasin (Promega), 20 U RNaseT$_1$ (Gibco BRL), BTEB2, or BTEB2ΔAD (~20 ng), and nuclear extracts (90 μg protein) containing template DNA (400 ng), and the control DNA (200 ng) which contains the adenovirus major late promoter and a 180 bp G-less cassette. After preincubation at 30°C for 25 min, transcriptions were initiated by the addition of a nucleotide mixture (600 μM ATP and CTP, 25 μM UTP, 300 μM 3′-*O*-methyl-GTP and 0.37 MBq [α-^{32}P]UTP), and incubated at 30°C for 45 min. Reactions were stopped by the addition of the stop buffer (0.5% SDS, 10 mM EDTA, 0.1 M sodium acetate, pH 5.2, 1

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mg/ml tRNA). Produced RNAs were extracted, precipitated with ethanol, and electrophoresed on an 8 M urea/6% polyacrylamide gel in 90 mM Tris-borate buffer, pH 8.2, containing 2.2 mM EDTA. Autoradiography and quantification of RNA were carried out with an Imaging analyzer (Fuji film, BAS1000).

Immunoblot Analysis—Immunoblot analysis was performed as described (*26*). Proteins were resolved by 13.5% SDS-PAGE and transferred to Nylon membranes (Hi-bond N,'Amersham). The membrane was probed with the first

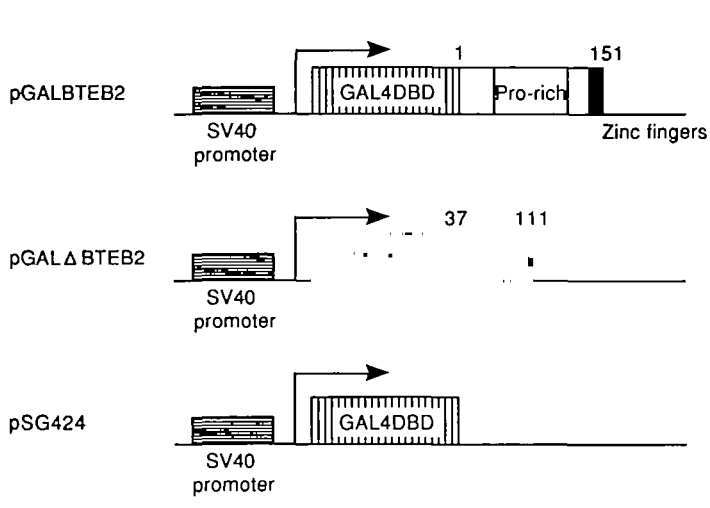

Fig. 1. **Structure and transcriptional activity of chimeric proteins containing a GAL4 DNA binding domain and a BTEB2 proline-rich domain.** Schematic structure of GAL4 DNA binding domain fused with BTEB2 lacking a part of zinc-finger domains or with the proline-rich domain are represented. The expression plasmids (4 μg) were introduced into COS cells with a reporter plasmid, pG$_5$EC (4 μg), and the expressed CAT activity was assayed. Values are averages of three separate experiments. A typical autoradiographic result is also shown below.

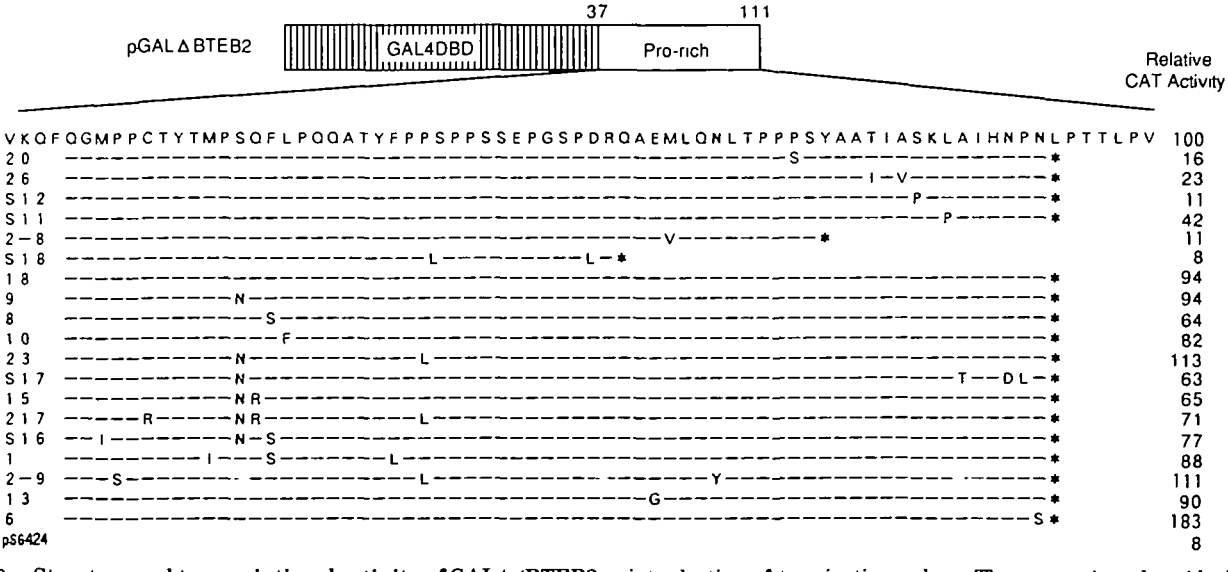

Fig. 2. **Structure and transcriptional activity of GAL4ΔBTEB2 with point mutations.** Point mutations were introduced into the proline-rich domain of GAL4ΔBTEB2 using a PCR technique. Only mutated amino acids are shown, while unchanged amino acids are shown by dashes. An asterisk shows C-terminal endpoints caused by introduction of termination codons. The expression plasmids (4 μg) with point mutations were introduced into COS cells with a reporter plasmid, pG$_5$EC (4 μg), and the expressed CAT activity was assayed. Values are averages of three separate experiments.

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antibody, raised against GAL4 DNA binding domain (*27*). Bands were visualized by means of alkaline phosphatase-linked secondary antibodies.

In Vitro Binding Assay—The glutathione S-transferase fusion proteins containing TBP, IIB, IIEα, IIEβ, IIFα, or IIFβ were isolated from *Escherichia coli* BL21(DE3)pLysS (Novagen) after induction with 0.2 mM isopropyl-1-thio-β-D-galactopyranoside at 37°C for 3 h. Bacterial extracts in 20 mM Tris buffer, pH 7.5, containing 150 mM NaCl, 1 mM EDTA, and 0.5% NP-40 were incubated with glutathione-Sepharose beads (Pharmacia) at 4°C for 1 h. After washing three times with the incubation buffer, the beads were incubated with 50 μl of the buffer containing the *in vitro* translated ^{35}S-labeled BTEB2 or BTEB2 mutant proteins at 4°C for 1 h. After washing three times with the buffer, the samples were eluted and subjected to 13.5% SDS-PAGE. Autoradiography and quantification of bound BTEB2 or its mutant proteins were carried out with an Imaging analyzer.

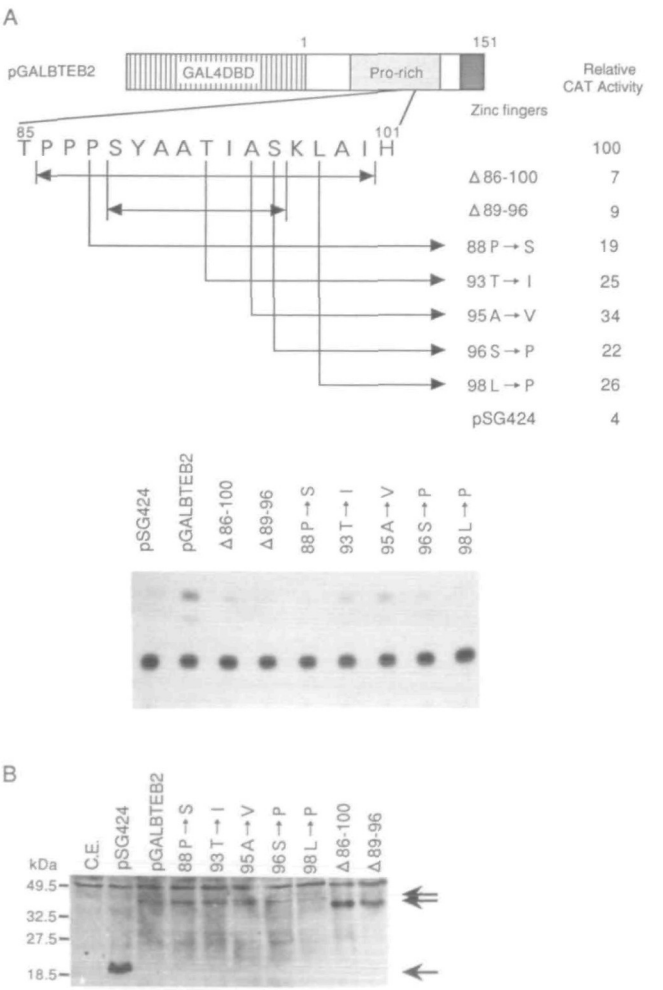

Fig. 3. **Point mutational and deletional analysis of the activation domain of BTEB2.** (A) Various expression plasmids containing replacement of amino acids that were identified to be important for transcriptional activity of BTEB2 in Fig. 2 were constructed by site-directed mutagenesis, and two expression plasmids which were deleted of short regions including the amino acids were also constructed. These expression plasmids (4 μg) were introduced into COS cells with a reporter plasmid, pG$_5$EC (4 μg), and the expressed CAT activity was assayed. Values are averages of three separate experiments. A typical autoradiographic result of the CAT activity is also shown below. (B) Immunoblot analysis was performed to examine the expression of the mutated or deleted GAL4BTEB2 using the anti-GAL4 antibody. Positions of the expressed normal proteins and proteins with point mutations are indicated by the upper arrow. Proteins with a deletion by the middle arrow. GAL4 DNA-binding protein by the lower arrow. The band near the top of each lane comes from non-specific immuno-reaction. C.E., whole cell extract from mock transfection.

RESULTS

Mutational Analysis of Transcriptional Activity of Proline-Rich Domain—Initially we investigated the transcriptional activity of BTEB2 by transfecting an expression plasmid pGALBTEB2 or pGALΔBTEB2 into COS cells together with a reporter plasmid, pG$_5$EC (*28*). GALBTEB2 and GALΔBTEB2 are chimeric proteins of GAL4 DNA binding domain fused with BTEB2 deleted of only a part of C-terminal zinc finger domains and with a sequence (amino acids 37 to 111) of BTEB2 covering most of the proline-rich region, respectively. The expression plasmid of GAL-BTEB2 enhanced markedly the CAT expression from the reporter gene containing 5 GAL4 recognition sites as shown in Fig. 1. The expressed CAT activity from pGALΔBTEB2 was more than half of that expressed from pGALBTEB2, suggesting that the proline-rich domain of BTEB2 retained most, if not all, of the transcription-enhancing activity of the BTEB2 transcription factor. To identify amino acid residues indispensable for the transcription-enhancing activity, we further examined the transcription-enhancing activity of various expression plasmids of GALΔBTEB2 with introduced point mutations. Point mutations were fairly randomly introduced in the proline-rich domain by a PCR technique, and the resultant amino acid replacements and termination codons were shown in Fig. 2. All the sequences listed in Fig. 2 except for two, S18 and 2-8 carried a mutation in a termination codon at the 104

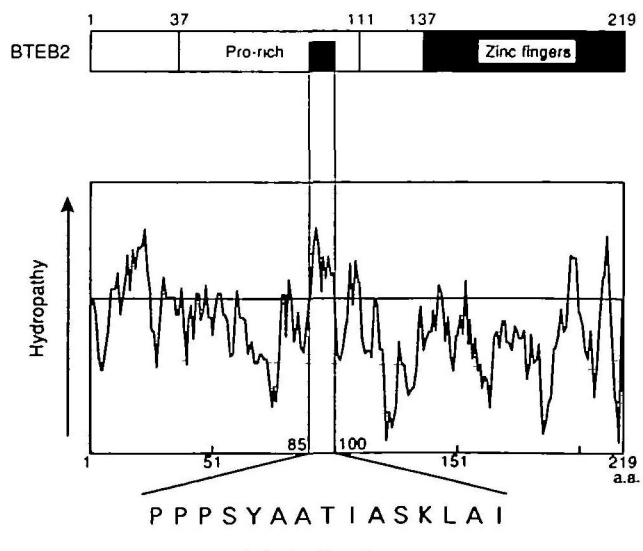

Fig. 4. **Hydropathy analysis of BTEB2.** The amino acid sequence of human BTEB2 was analyzed for hydrophobicity and hydrophilicity according to Kyte and Doolittle (*36*).

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position, suggesting that these sequences were derived from one with termination codon like mutant 18 by undergoing further mutation. However, this mutation at the 104 did not significantly affect the transcriptional activity of the activation domain (compare the CAT activity of mutant 18 to that of wild type). Amino acids whose mutation markedly decreased the CAT activity were clustered in a short segment from amino acid No. 88 to 98, whereas mutations occurring in amino acids from the 43 to 83 position showed essentially no effect on the activity. In agreement with these results, the CAT activity of truncated proteins (mutants 2-8 and S18) by introduction of stop codons at the 90 and 76 position, respectively, was reduced almost to the basal level of pSG424. We further introduced point mutations as found in mutants 20, 26, S11, and S12 to pGALBTEB2 and assayed the expressed CAT activity as shown in Fig. 3. All the mutations markedly decreased the CAT activity, in confirmation of the above results. Interestingly, mutations with an additional proline residue as a substituent (Ser96 and Leu98 to Pro) reduced the CAT expression. Two short deletions in the region reduced the CAT activity nearly to the basal level (Fig. 3A). The expression of mutated proteins in the cells were examined by the immunoblot analysis (Fig. 3B). Proteins with a deletion were expressed abundantly in the cells. Two mutated proteins (Ser96 and Leu98 to Pro) were relatively weakly expressed. These results indicate that low transcription-enhancing activity of these mutated proteins was not due to low expression levels of proteins in the transfected cells, although we could not rigorously exclude the possibility that the low level of expression of two proteins (Ser96 and Leu98 to Pro) may partly explain the low

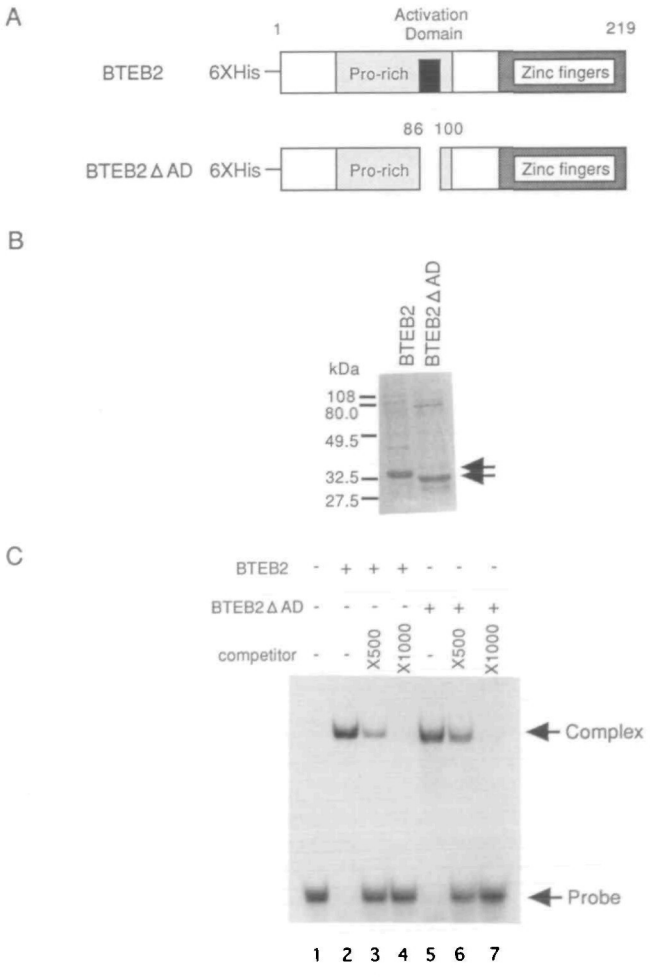

Fig. 5. **DNA binding activity of BTEB2 and its deletion mutant expressed in the baculovirus system.** Structure of BTEB2 and BTEB2 with deletion of the activation domain (BTEB2⊿AD) produced in the baculovirus expression system was schematically represented (A). Both proteins were partially purified by the Ni-affinity chromatography, and analyzed for their purity by 10% SDS-PAGE (B). The GC box-binding activity of the two proteins were analyzed by the gel mobility shift assay (C). Lane 1, no protein; lane 2-4, BTEB2; lane 5-7, BTEB2⊿AD. Unlabeled oligonucleotides, 500- (lanes 3 and 6) or 1,000-fold (lanes 4 and 7) were used as a competitor.

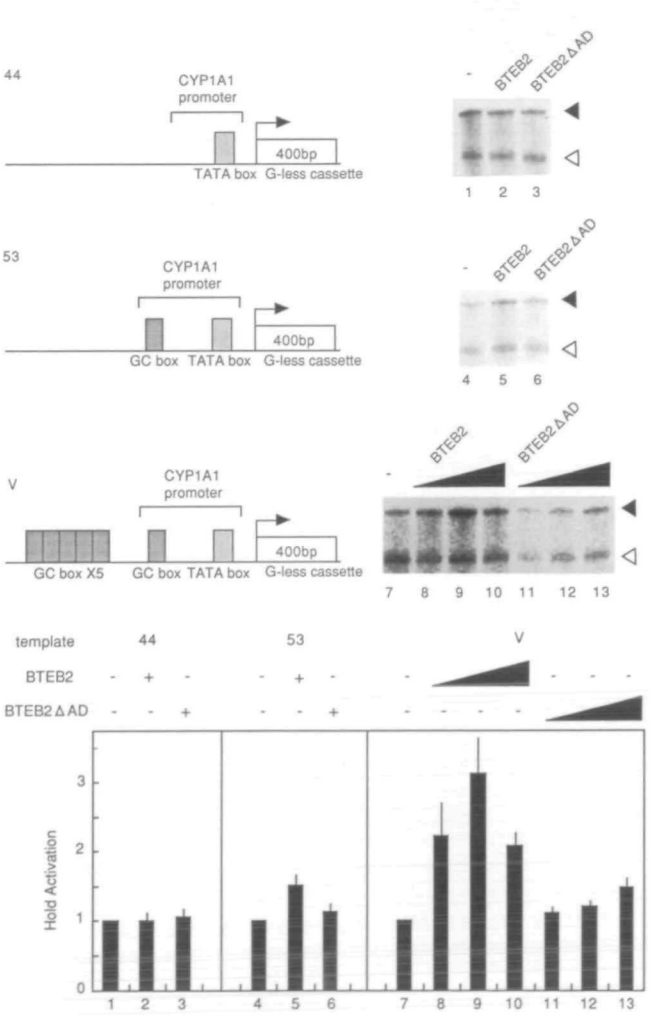

Fig. 6. ***In vitro* transcription-enhancing activity of BTEB2.** *In vitro* transcription was carried out using purified BTEB2 and BTEB2 ⊿AD and nuclear extracts of Sf9 insect cells. Synthesized RNAs were extracted, precipitated, and subjected to 8 M urea-12.5% polyacrylamide gel electrophoresis. Positions of migrated products from experimental and reference promoters (adenovirus major late promoter) are indicated by filled and open arrowheads, respectively. Schematic structure of templates used and typical autoradiographic results are shown to the upper-left and upper-right, respectively. Synthesized RNAs were quantified on an Imaging analyzer, and shown by a histogram below. The values represent the mean ± SD of three separate determinations, and were normalized using the values of lane 1, 4, or 7.

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transcription-enhancing activity of the proteins. Taken together, these results strongly suggest that this short segment is indispensable for the transcription-enhancing activity of BTEB2. Figure 4 shows hydropathy profile of the BTEB2 protein. The transactivation region identified was one of the hydrophobic regions rich in proline and alanine residues.

Transcriptional Activity of BTEB2 in an In Vitro Transcription System—To confirm the importance of the activation domain defined above, we purified BTEB2 from the baculovirus expression system, and investigated its activity in an *in vitro* transcription system with nuclear extracts from insect cells of Sf9 (*21*) lacking GC box-binding proteins. Figure 5 shows the structure (A) and purity (B) of BTEB2 and its derivative without the transactivation domain (BTEBΔAD) used for the experiments. Both proteins were purified to a similar extent (more than 80%) by the Ni-affinity chromatography. The DNA binding capacity of the purified proteins to GC box was examined by the gel mobility shift assay as shown in Fig. 5C. The shifted bands due to the binding of the two proteins were single, and was similarly competed out by addition of the unlabeled oligonucleotides used as the probe (Fig. 5C), suggesting that the binding capacities of the two proteins were similar

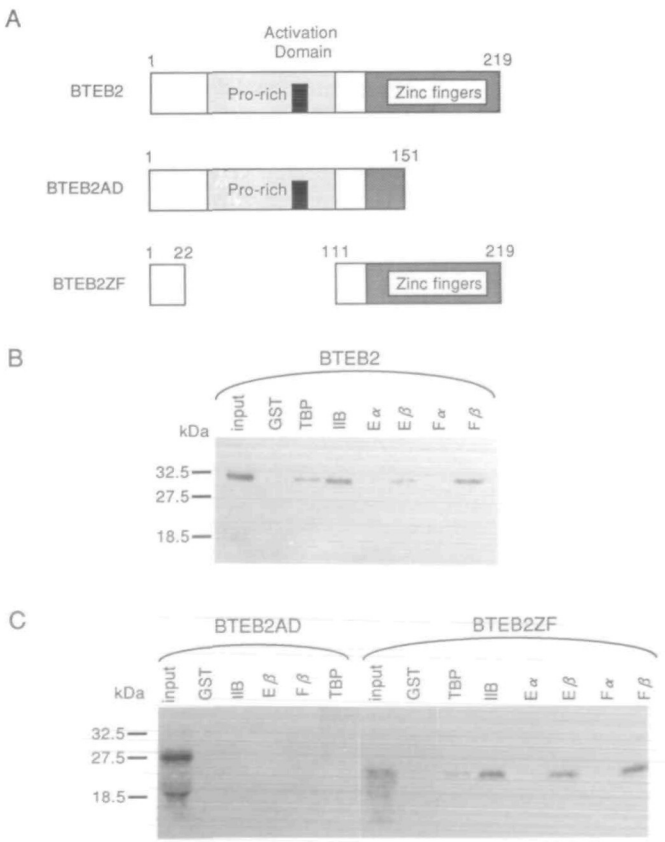

Fig. 7. **Binding of basic transcription factors to BTEB2.** Structure of BTEB2 and two deletion mutants, BTEB2AD and BTEB2ZF, containing proline-rich domain and zinc finger domains, respectively, are schematically represented. The ^{35}S-labeled proteins synthesized in the cell free system were mixed with bacterially expressed basic factors fused to glutathione S-transferase. Bound BTEB2, BTEB2AD, or BTEB2ZF was then washed, eluted, and analyzed by 12.5% SDS-PAGE.

to each other, and that the deletion in the proline-rich domain did not affect the DNA binding activity of BTEB2. We performed *in vitro* transcription experiments using the purified proteins and several templates with or without GC boxes in the promoter region. By changing various parameters such as concentrations of DNA, magnesium ion, and nuclear proteins, we finally optimized the condition of transcription as described in "MATERIALS AND METHODS." As shown in Fig. 6, addition of BTEB2 to the reaction mixture exhibited no effect on transcription with template lacking GC box (template 44). When one GC box was contained in the promoter region of the template (template 53), a weak but reproducible enhancement (1.6-hold) was observed by the addition of BTEB2 but not BTEB2ΔAD. When a template containing six GC boxes (template V) was used for the transcription experiments, a marked increase in the transcription was observed by the addition of BTEB2 (Fig. 6, lanes 8 and 9). Further addition of BTEB2 repressed the transcription probably due to sequestering of basic transcription factors (Fig. 6, lane 10). Addition of BTEB-2ΔAD showed only a marginal increase in the transcription (Fig. 6, lanes 12 and 13). These results clearly demonstrated that BTEB2 is a transcription activator by binding to the GC box, and that the short hydrophobic segment identified in the *in vivo* experiments is also important for the transcriptional activation *in vitro*.

Interaction of BTEB2 with Initiation Factors—We examined interaction of BTEB2 with transcription initiation factors, TBP, TFIIB, TFIIEα, TFIIEβ, TFIIFα, and TFIIFβ. These factors are known to interact with activation domains of several transcription factors (*29-32*). Of these transcription factors, TFIIB, TFIIEβ, and TFIIFβ strongly interacted with BTEB2, while TBP did weakly as shown in Fig. 7. We divided BTEB2 into two portions: one is mainly composed of the proline-rich region containing the transcription activation domain, while the other is mainly composed of the zinc finger region. We examined the binding activity of these fragments with the basic transcription factors. As shown in Fig. 7C, the binding activity was carried by the region mainly composed of the zinc finger domains. No factors examined interacted with the proline-rich domain.

DISCUSSION

By introducing point mutations and small deletions into the proline-rich domain, we have identified a short segment indispensable for transcriptional activation of BTEB2. This region is rich in proline and alanine residues, and overlaps one of the hydrophobic regions of BTEB2 as shown in Fig. 4. Since some amino acid changes (such as Ala95 to Val, Fig. 3) which are presumed to cause a minimal change in the conformation of proteins, largely decreased the transcriptional activity, the role of the hydrophobic region is not simply to keep a conformation of the protein. The experiments using mutants shown in Fig. 3 also suggest that the hydrophobicity of the region was not simply the determinant for the transcription-enhancing activity because a few amino acid changes which increased hydrophobicity (for example, Thr93 to Ile) markedly reduced the transcription-enhancing activity. These results strongly suggest that the context of the sequence was equally important. Analysis of transcriptional activation domain of Sp1 (*33*) and

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BTEB (*15*), containing glutamine-rich and non-typical activation domains, respectively, revealed that hydrophobic sequences in the activation domain were important for the transcriptional activity of the two factors. We examined the sequence of EKLF and LKLF for similar hydrophobic sequences in their presumed activation domains because the two factors and BTEB2 share common characteristics such as tissue- or cell type-specific expressions and transactivation of genes presumably by means of the proline-rich domains (*8, 9*). In addition to these resemblances, the three proteins show a highest sequence similarity in the zinc finger domains among GC-box proteins (*9*). The regions of amino acid 190-250 for EKLF and 200-240 for LKLF were found to possess both hydrophobicity and high content of proline and alanine residues. Although it is necessary to clarify experimentally that these regions are required for the transcriptional activity, these regions may commonly serve as a transactivation surface as observed with BTEB2.

To find the target factor(s) which directly interacts with BTEB2, we attempted an *in vitro* binding assay between BTEB2 and basic transcription factors including TBP, TFIIB, TFIIE, and TFIIF. As shown in Fig. 7, BTEB2 interacted strongly with TFIIB, TFIIEβ (a subunit of TFIIE), and TFIIFβ (a subunit of TFIIF), while only a weak interaction was observed with TBP. These interactions were not attributed to the proline-rich domain. These results suggest that there are multiple interactions between BTEB2 and basic transcription factors to form a stable preinitiation complex as exemplified with VP16 (*30*) and Krüppel (*31*). It is important to find a factor which interacts with the activation domain of BTEB2. Some TAFs might interact with it since it is reported that activation domain of Sp1 and VP16 bind TAF110 (*34*) and TAF40 (*35*), respectively.

EKLF and LKLF are elucidated or supposed to be involved in β-globin synthesis. These factors could strongly transactivate the β-globin gene in cultured cells (*8, 9*) and in developing mouse embryos (*17, 18*). It is difficult to assume, however, that the β-globin or other globin genes is a target gene of BTEB2 because the expression pattern of the BTEB2 mRNA is quite different from that of globin genes. Preliminary investigation of the expression pattern of the BTEB2 mRNA in the mouse embryos demonstrated that the BTEB2 mRNA was not expressed in the fetal liver and other tissues for embryonic hematopoiesis (data not shown). Although a small amount of the expression below the detection level could not be excluded, these data along with the result that no expression was detected in spleen of adult rats (*7*) suggest that BTEB2 is not involved in β-globin synthesis. It remains to be seen which gene is a target for BTEB2.

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