HBZ interacts with JunD and stimulates its transcriptional activity

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Abstract Human T-cell leukemia virus type I (HTLV-I) bZIP factor (HBZ) is a viral basic leucine zipper protein that was originally described as a partner of cAMP response element binding protein-2 and as a repressor of HTLV-I viral transcription. In addition, HBZ is able to interact with the activator protein-1 (AP-1) transcription factors c-Jun and JunB, the interaction with c-Jun leading to a transcriptional repression of AP-1-regulated genes. Here we show that HBZ also interacts with JunD in vitro and in vivo, and that this association occurs via the bZIP domain of the two proteins. Moreover, we show that HBZ can activate JunD-dependent transcription and that its amino-terminus is required.

Key words: Human T-cell leukemia virus type I; Basic leucine zipper factor; Human T-cell leukemia virus type I basic leucine zipper; Activator protein-1; JunD

1. Introduction

Human T-cell leukemia virus type I (HTLV-I) is the etiologic agent of an aggressive and fatal T-cell malignancy, adult T-cell leukemia (ATL), and is also associated with the development of a variety of chronic inflammatory diseases. HTLV-I encodes a 40-kDa protein, Tax, which has multiple functions in the development of ATL [1,2]. Tax activates and represses a large number of cellular genes, which could contribute to the phenotype of the infected cells and to the subsequent development of the diseases caused by HTLV-I. Tax activates the transcription of viral and cellular genes through selective enhancer elements such as the cAMP-responsive element (CRE) [3], the nuclear factor-κB element [4], and the CArG box [5]. Tax can also activate the activator protein-1 (AP-1) binding sites in T-cells [6]. Moreover, HTLV-I-transformed T-cell lines exhibit high expression levels of mRNAs encoding various AP-1 transcription factor family members, such as c-Jun, JunB, JunD, c-Fos and Fra-1. Four of these factors (c-Jun, JunD, c-Fos and Fra-1) are directly induced by Tax in the human T-cell line Jurkat [7]. Thus, AP-1 is one of the candidates involved in the dysregulated phenotypes of T-cells infected with HTLV-I.

The transcription factor AP-1 is composed primarily of members of the Jun (c-Jun, JunB and JunD) and Fos (c-Fos, FosB, Fra-1 and Fra-2) families of proteins. These proteins are characterized by a highly charged, basic DNA-binding domain immediately adjacent to an amphipathic dimerization domain referred to as the 'leucine zipper' and constituted of a heptad repeat of leucine residues [8]. They are part of the so-called bZIP family (basic leucine zipper) of transcription factors. Jun proteins can form both homo- and heterodimers among themselves. Jun proteins also dimerize with Fos proteins and other bZIP factors such as members of the activating transcription factor/CRE-binding protein (ATF/CREB) family and the Maf transcription factors. Dimerization is required for efficient interaction with specific DNA sequences found in the promoter of various target genes. These target genes are important for regulating many biological processes including proliferation, differentiation, apoptosis and transformation [9–11].

In the present study, we examine the interaction of one of the AP-1 family members, JunD, with the HTLV-I bZIP factor HBZ. This protein contains an amino-terminal transcriptional activation domain and a leucine zipper motif in its carboxy-terminus [12]. HBZ acts as a repressor of Tax-induced viral transcription by forming heterodimers with the transcription factor CREB-2 that are no longer able to bind to the viral CRE and consequently are no longer able to activate the HTLV-I promoter [12]. Moreover, HBZ is able to interact with the AP-1 factors c-Jun and JunB. The interaction of HBZ with c-Jun results in the decrease of c-Jun DNA-binding activity and prevents this protein from activating the transcription of AP-1-regulated genes and the basal expression of the HTLV-I promoter [13]. On the other hand, the combination of HBZ with JunB has a slightly higher transcriptional activity than JunB alone [13]. Our results indicate that HBZ and JunD also interact in vitro and in vivo, associating via their bZIP domain. In addition, we show that HBZ can activate JunD-dependent transcription in CEM cells and that its amino-terminus is required.

2. Materials and methods

2.1. *Glutathione* S-*transferase (GST) pull-down assay*

The complete JunD coding sequence was cloned into pGEX4-T2 (Amersham Biosciences, Buckinghamshire, UK) after amplification by polymerase chain reaction (PCR) from pCMV-JunD-Flag. HBZ cDNA cloned in pCi-neo [12] was transcribed and translated in the presence of [^{35}S]methionine using a TNT T7-coupled reticulocyte lysate system (Promega, Madison, WI, USA), and incubated at 4°C

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Abbreviations: HTLV-I, human T-cell leukemia virus type I; ATL, adult T-cell leukemia; CREB-2, cAMP response element binding protein-2; AP-1, activator protein-1; bZIP, basic leucine zipper; HBZ, HTLV-I bZIP factor

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with equal amounts of GST or GST-JunD immobilized on glutathione Sepharose beads (Bulk GST purification module of Amersham Biosciences) in a buffer containing 50 mM Tris–HCl pH 7.4, 120 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40. After 2 h incubation, the beads were washed three times with the incubation buffer, and five times with a buffer containing 50 mM Tris–HCl pH 7.4, 1 M NaCl, 5 mM EDTA, 0.5% Nonidet P-40. The bound proteins were then analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by autoradiography.

2.2. Immunoprecipitation and Western blot assays

HBZ cDNA was generated by PCR amplification on pCI-HBZ [12], digested by *Bam*HI and *Hin*dIII, and subcloned into pcDNA3.1(-)/Myc-His (Invitrogen, Carlsbad, CA, USA). For immunoprecipitation of HBZ, the plasmids pcDNA3.1(-)/Myc-His-HBZ and pCMV-JunD-Flag were cotransfected into HEK 293T cells using the FuGENE 6 transfection reagent as described by the Roche Molecular Biochemicals protocol (Indianapolis, IN, USA). Cultures were grown for 48 h in Dulbecco's modified Eagle's medium (Cambrex, Verviers, Belgium) supplemented with a 1% penicillin and streptomycin antibiotic mixture (Life Technologies, Eragny, France) and 10% fetal calf serum (Life Technologies). After centrifugation, cells were lysed in a buffer containing 50 mM Tris–HCl pH 7.4, 120 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, and proteins were immunoprecipitated from protein extracts (0.8 mg of total proteins) using an anti-HBZ rabbit serum [12]. Bound fractions were then electrophoresed onto a SDS–PAGE and analyzed by immunoblotting as described [14]. The mouse anti-Flag was purchased from Sigma (St. Louis, MO, USA). In the case of C8166, HUT102 and MT4 cells, proteins were immunoprecipitated from 2.5 mg of total proteins and the protocol used was the same as above. The polyclonal goat anti-JunD was purchased from Santa Cruz Biotechnologies (Santa Cruz, CA, USA).

2.3. Yeast two-hybrid system

The complete and bZIP-deleted JunD coding sequences were cloned in pGAD10 (Clontech, Palo Alto, CA, USA) after amplification by PCR from pCMV-JunD-Flag. The *Saccharomyces cerevisiae* HF7c yeast cells were cotransformed with an expression vector containing either the entire coding sequence of JunD or the coding sequence of JunD with the bZIP domain deleted, each one fused to the GAL4 activation domain (pGAD-JunD and pGAD-JunD ΔZIP respectively), together with plasmids expressing either the GAL4 DNA-binding domain alone (pGBT9) or fused to lamin (pGBT9-lamin) or fused to HBZ bZIP (pGBT9-HBZ bZIP) [13]. pGBT9-HBZ bZIP was also cotransformed with a plasmid expressing the GAL4 activation domain alone (pGAD). The β-galactosidase assay with *o*-nitrophenyl-β-D-galactoside (ONPG) as substrate was carried out as described in the Clontech protocol.

2.4. Immunofluorescence microscopy analysis

COS7 cells were transfected using the FuGENE 6 transfection reagent with 2 μg of the plasmid pEGFP-HBZ expressing a GFP (green fluorescent protein)-HBZ fusion protein and 2 μg of the plasmid pDsRed1-JunD expression an RFP (red fluorescent protein)-JunD fusion protein. pEGFP-HBZ was described previously [12]. To obtain pDsRed1-JunD, the complete JunD coding sequence was cloned in pDsRed1-N1 (Clontech) after amplification by PCR from pCMV-JunD-Flag. Cells were cultivated on glass slides and then analyzed by fluorescence 40 h after transfection. Analysis of green, red and merged fluorescence was performed with a Leica DC250 immunofluorescent microscope.

2.5. Transfections and luciferase assays

The lymphoblastoid CEM cell line was obtained from the American Type Culture Collection (Bethesda, MD, USA). Cells were cultured in RPMI 1640 medium (Cambrex) supplemented with a 1% penicillin and streptomycin antibiotic mixture and 10% fetal calf serum to a density of 5×10^5 cells/ml in a 5% CO_2 atmosphere. CEM cells were transiently cotransfected according to the previously published procedure [15]. 5 μg of pcDNA3.1-lacZ (β-galactosidase-containing reference plasmid) was included in each transfection to control the transfection efficiency. The total amount of DNA in each series of transfections was equal, the balance being made with empty vectors. Cell extracts equalized for protein content were used for luciferase and β-galactosidase assays. The activation domain-deleted HBZ coding sequence, HBZ ΔAD, was cloned in pcDNA3.1(-)/Myc-His after amplification by PCR from pcDNA3.1(-)/Myc-His-HBZ.

2.6. Microwell colorimetric AP-1 assay

Nuclear cell extracts (15 μg) were incubated with 30 μl of binding buffer (10 mM HEPES pH 7.5, 8 mM NaCl, 12% glycerol, 0.2 mM EDTA, 0.1% bovine serum albumin) in microwells coated with probes containing the AP-1 site (Trans-AM™ AP-1 of Active Motif Europe, Belgium). After 1 h incubation at room temperature, microwells were washed three times with phosphate-buffered saline+0.1% Tween 20. The AP-1-bound complexes were detected with a goat anti-JunD antibody and a peroxidase-conjugated anti-goat antibody. For the colorimetric detection, tetramethylbenzidine was incubated at room temperature before adding stopping solution. Optical density was read at 450 nm, using a 620 nm reference wavelength with a Tecan microplate reader.

3. Results and discussion

HBZ is a viral protein that interacts in vitro and in vivo with the AP-1 transcription factors c-Jun and JunB [13]. It was therefore interesting to investigate if HBZ could also interact with the third Jun family member, JunD, and what would be the effect of HBZ on JunD-dependent transcription. First, we analyzed the interaction between HBZ and JunD in vitro using recombinant proteins. A fusion protein of JunD with GST was produced in *Escherichia coli* and the binding to [^{35}S]methionine-labeled HBZ produced in rabbit reticulocyte lysate was analyzed. As shown in Fig. 1A, [^{35}S]HBZ bound to GST-JunD (lane 3) but not to GST alone (lane 2). This result demonstrates that HBZ and JunD interact in vitro.

To confirm the in vivo relevance of the interaction between HBZ and JunD, we coexpressed the two proteins in HEK 293T cells. Cell extracts were then immunoprecipitated with either a rabbit anti-HBZ antiserum or a preimmune serum from the same rabbit [12], followed by Western blot analysis using an anti-Flag monoclonal antibody, JunD being flagged. By this approach, JunD was found in the immunoprecipitate with the anti-HBZ antiserum (Fig. 1B, lane 3) but not in the control immunoprecipitate (Fig. 1B, lane 2). When the same experiment was performed with extracts from HEK 293T cells transfected only with JunD, no protein was found in the immunoprecipitate with the anti-HBZ antiserum (Fig. 1B, lane 5), confirming the specificity of the association between HBZ and JunD.

To confirm that endogenous proteins could also interact in infected cells, we performed an immunoprecipitation, with the anti-HBZ antiserum, from the cell lysate of three different HTLV-I-infected cell lines, C8166, HUT102 and MT4. We have already demonstrated that HBZ can be detected in the C8166 and MT4 cells following an anti-HBZ immunoprecipitation [12]. The proteins found in the immunoprecipitate with the anti-HBZ antiserum were then analyzed with a polyclonal anti-JunD antibody. This approach revealed the presence of a complex between HBZ and JunD in C8166 extracts (Fig. 1C, lane 2) and in MT4 extracts (data not shown). In addition, such a complex was also detected by the same approach in HUT102, another HTLV-I-infected cell line for which nothing has been demonstrated (Fig. 1C, lane 4). Altogether, these results clearly demonstrate that HBZ and JunD interact in vivo.

In the analysis of the interaction of HBZ with either c-Jun or JunB, it has been demonstrated that the bZIP domain of the proteins was involved in the association [13]. To investi-

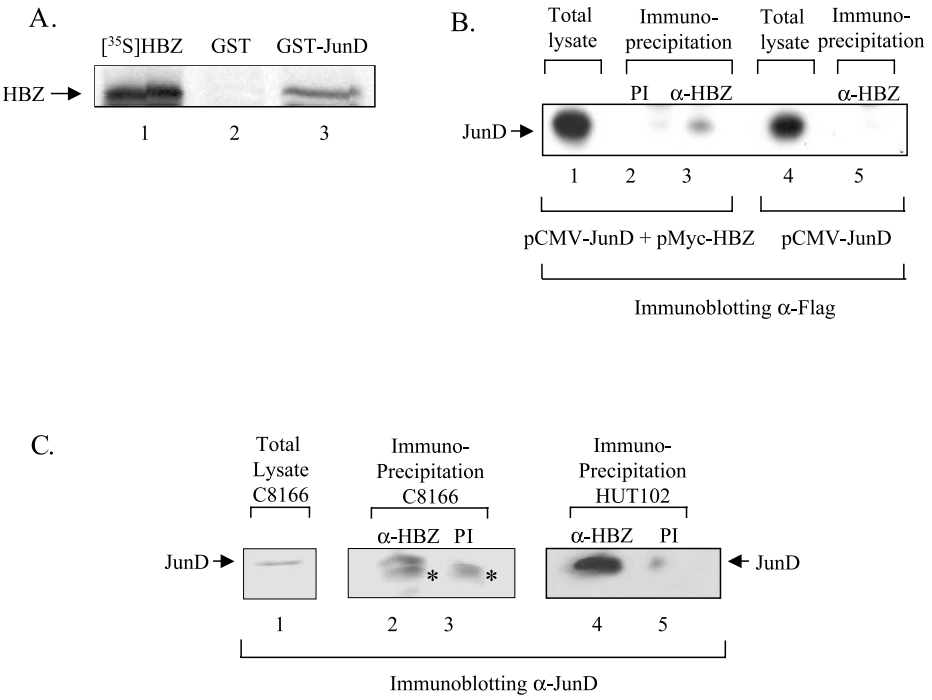

Fig. 1. A: Binding of HBZ to JunD in vitro. GST protein and GST-JunD fusion protein were purified from *E. coli*. In vitro translated HBZ in the presence of [^{35}S]methionine (lane 1) was incubated with equal amounts of GST protein (lane 2) or GST-JunD fusion protein (lane 3) immobilized on glutathione Sepharose beads. After incubation, the bound proteins were analyzed by SDS–PAGE followed by autoradiography. B: Binding of HBZ to JunD in vivo. HEK 293T cells were transfected with the HBZ expression vector pcDNA3.1(-)/Myc-His-HBZ (lanes 1–3) or the corresponding empty vector (lanes 4 and 5) and the plasmid pCMV-JunD-Flag (lanes 1–5). Proteins from total lysates were directly probed with mouse anti-Flag (lanes 1 and 4) or were immunoprecipitated with rabbit anti-HBZ (lanes 3 and 5) or preimmune serum (lane 2), followed by Western blot analysis with anti-Flag (lanes 2, 3 and 5). C: Binding of HBZ to JunD in HTLV-I-infected cells. Proteins from the total lysates of the HTLV-I-infected cells C8166 and HUT102 were also immunoprecipitated with rabbit anti-HBZ (lanes 2 and 4) or preimmune serum (lanes 3 and 5), and immunoprecipitated proteins were analyzed with goat anti-JunD (lanes 2–5). Lane 1 corresponds to the total lysate of C8166 cells directly probed with anti-JunD. The asterisk indicates a non-specific band. D: Analysis of the interaction between HBZ and JunD in the yeast two-hybrid system. Yeasts were cotransformed with an expression vector containing either the entire coding sequence of JunD or the coding sequence of JunD with the bZIP domain deleted, each one fused to the GAL4 activation domain (pGAD-JunD and pGAD-JunD ΔZIP respectively), together with plasmids expressing either the GAL4 DNA-binding domain alone (pGBT9) or fused to lamin (pGBT9-lamin) or fused to HBZ bZIP (pGBT9-HBZ bZIP). pGBT9-HBZ bZIP was also cotransformed with a plasmid expressing the GAL4 activation domain alone (pGAD). The β-galactosidase assay with ONPG as substrate was carried out on three independent colonies per transformation as described in the Clontech protocol. The mean values expressed in Miller units are shown.

gate the possibility that the bZIP domains of HBZ and JunD are also involved in the association of the two proteins, we produced a truncated mutant of JunD by removing the leucine zipper, and this mutant was analyzed by using the yeast two-hybrid system as already described [16]. For this analysis, the full-length product of JunD and its mutated form were fused at their amino-termini to the activation domain of the yeast transcription factor GAL4 (pGAD-JunD and pGAD-JunD ΔZIP respectively) and were tested in *S. cerevisiae* in the presence of the HBZ bZIP domain fused at its amino-terminus to the GAL4 DNA-binding domain (pGBT9-HBZ bZIP). As shown in Fig. 1D, JunD was no longer able to interact with HBZ when its leucine zipper was deleted. Moreover, to be sure of the specificity of the test, JunD was also tested in the presence of an unrelated protein (pGBT9-lamin) or in the presence of the GAL4 DNA-binding domain alone (pGBT9). In these conditions, no significant β-galactosidase activity was detected (Fig. 1D).

To confirm the relevance of the interaction between HBZ and JunD in vivo, we studied the localization of both proteins, alone or in conjunction, inside the cells by immunofluorescence microscopy. However, the anti-HBZ serum used for the immunoprecipitation experiments described above does not work for immunofluorescence experiments. Thus, since the endogenous HBZ protein cannot be detected by the anti-HBZ antiserum, we performed the experiment on cells transfected with vectors encoding tagged proteins. For this purpose, COS7 cells were transiently cotransfected with vectors encoding HBZ tagged with GFP, JunD tagged with RFP, and the corresponding empty vectors pEGFP and pDs-Red1. COS7 cells were used in this experiment for their capacity to spread out and to give a precise GFP signal. When HBZ-GFP was transfected in the absence or presence of JunD, we found that HBZ tagged with GFP exhibited a granular nuclear distribution (Fig. 2a,g) as already described [12]. On the other hand, when JunD was transfected in the absence of HBZ, it localized in the nucleus with a diffuse pattern (Fig. 2e). But when JunD was transfected in the presence of HBZ, it was relocalized to the same nuclear spots as HBZ. The colocalization of HBZ and JunD in the nucleus was visualized in yellow color (Fig. 2i) corresponding to the merging of the green fluorescence of HBZ-GFP fusion protein (Fig. 2g) and the red staining of JunD-RFP fusion protein (Fig. 2h). These observations support the notion that HBZ and JunD colocalize in the nucleus and also show that HBZ entails an intranuclear redistribution of JunD. This had already been described for c-Jun and JunB [13].

We next examined the effect of HBZ on the transcription from the collagenase promoter that contains a canonical AP-1 element. For this purpose, transient cotransfection assays were carried out using a luciferase reporter gene driven by the collagenase promoter. The transfections were performed in CEM cells in the absence or the presence of JunD and increasing amounts of HBZ (Fig. 3A). As expected, JunD was found to activate the expression of the luciferase reporter gene by about 16-fold. But contrary to the results obtained with c-Jun [13], in this case the luciferase activity was overstimulated in the presence of HBZ. This stimulation was proportional to the quantity of transfected HBZ plasmid. Then, we examined the effect of HBZ on JunD DNA-binding activity. JunD homodimers are known to have very weak DNA binding in vitro [17]. For this reason, using a microwell colorimetric assay, we analyzed JunD DNA-binding activity in the presence or absence of HBZ. Nuclear cell extracts of HEK

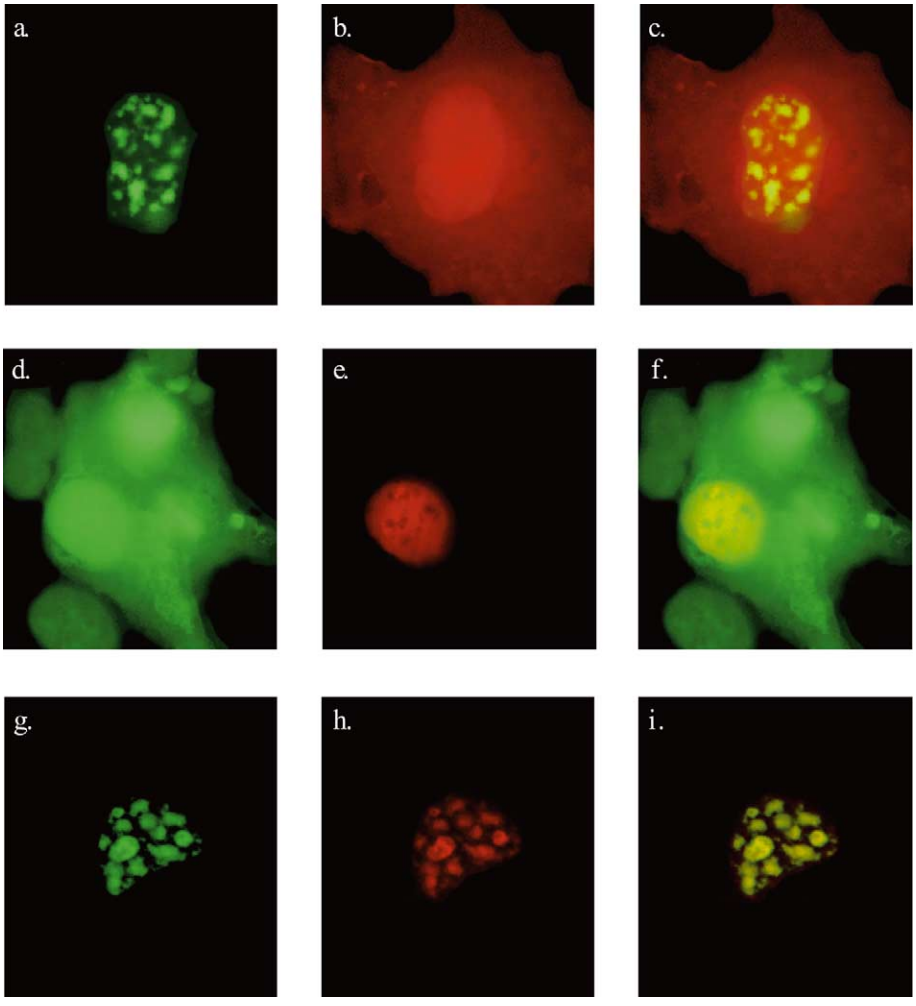

Fig. 2. Immunofluorescence microscopy analysis of the colocalization of HBZ and JunD in vivo. COS7 cells were cotransfected with pEGFP-HBZ and pDs-Red1 (a–c), pEGFP and pDs-Red1-JunD (d–f) or pEGFP-HBZ and pDs-Red1-JunD (g–i). Analysis of the green (a,d,g), red (b,e,h) and merged (c,f,i) fluorescence was performed by immunofluorescence microscopy.

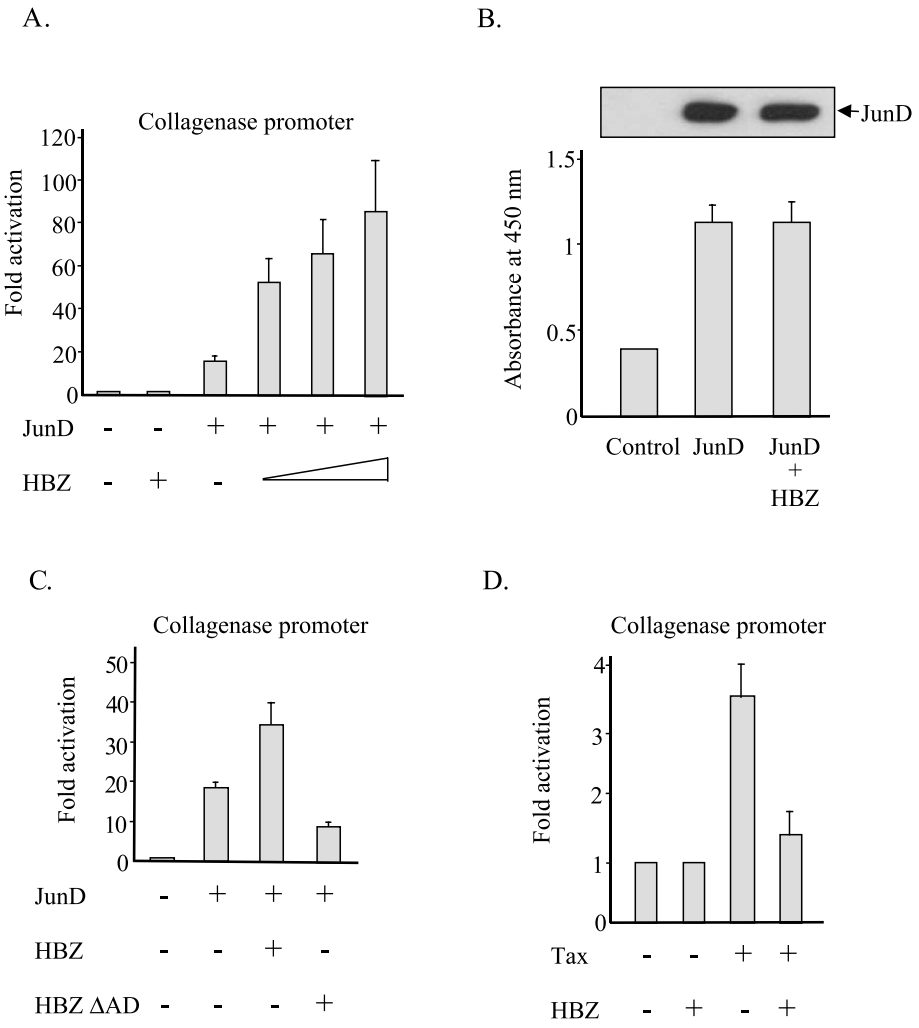

Fig. 3. A: Activation of JunD transactivation by HBZ. CEM cells were cotransfected with 2 μg of luciferase gene driven by the collagenase promoter, 5 μg of pcDNA3.1-lacZ (β-galactosidase containing reference plasmid), 0 or 1 μg of JunD expression plasmid (pCMV-JunD-Flag) and pcDNA3.1(-)/Myc-His-HBZ (0, 1, 2 and 3 μg). The total amount of DNA in each series of transfections was equal, the balance being made with empty plasmids. The luciferase values were normalized for β-galactosidase activity and expressed as fold relative to that of cells transfected with pcDNA and pCMV in the presence of the luciferase reporter gene. Values represent the mean ± S.D. ($n = 3$). B: HBZ does not alter JunD DNA-binding activity on AP-1 sites. Microwells containing the DNA probe with the AP-1 site were incubated with nuclear cell extracts of HEK 293T cells transfected by JunD alone (3 μg of pCMV-JunD-Flag) or associated with HBZ (3 μg of pcDNA3.1(-)/Myc-His-HBZ). The negative control corresponds to cells transfected by the empty plasmids. The data represent the means of three values ± S.D. C: The activation domain of HBZ is required for the stimulation of the collagenase promoter. CEM cells were cotransfected as described in the legend of A, but with 1 μg of pcDNA3.1(-)/Myc-His-HBZ and 1 μg of pcDNA3.1(-)/Myc-His-HBZ ΔAD. Values represent the mean ± S.D. ($n = 3$). D: Effect of HBZ on Tax-mediated AP-1 transcription. CEM cells were cotransfected as described in the legend of A, but with 0 or 1 μg of Tax expression plasmid (pSG-Tax) and 0 or 1 μg of pcDNA3.1(-)/Myc-His-HBZ. Values represent the mean ± S.D. ($n = 3$).

293T cells transfected by JunD alone or associated with HBZ were incubated in the presence of a double-stranded oligonucleotide containing the AP-1 site, TGAGTCA, immobilized in microwell plates. JunD specifically bound to this oligonucleotide was then detected using an anti-JunD antiserum. As shown in Fig. 3B, HBZ did not significantly alter JunD binding to the AP-1 site.

In order to explain the effect of HBZ on JunD-mediated transcription and since HBZ has been described to contain a potential activation domain located in its amino-terminus [12], we postulated that this domain could be involved in this regulation. To find out if this was the case, we constructed a mutated HBZ (HBZ ΔAD) deleted of its 80 first amino acids. First, we verified that the cellular localization of this mutant was comparable to that of wild-type HBZ (data not shown). Then, CEM cells were cotransfected with JunD and HBZ ΔAD as described above. The luciferase activity was repressed in the presence of HBZ ΔAD (Fig. 3C). This result suggests not only that the amino-terminus of HBZ is necessary for HBZ to stimulate JunD-dependent transcription but also that HBZ ΔAD could probably dimerize with JunD to antagonize its action. This mechanism may not be the only one to be claimed in order to explain the effect of HBZ on JunD-mediated transcription. It is also possible that HBZ might act positively on JunD-mediated transcription by preventing this AP-1 factor from recruiting co-repressors, for example menin [18,19]. Further experiments will be required to test this hypothesis.

HBZ is a viral protein known to repress HTLV-I viral transcription by interacting and forming unstable complexes on

the CRE sequences with the transcription factors CREB-2 and c-Jun. HBZ also represses c-Jun-dependent AP-1-regulated gene transcription. Here, we show for the first time that HBZ can stimulate in a dose-dependent manner the transcription by associating with a bZIP factor, JunD. It is particularly interesting to note that HBZ has a different and opposite action on c-Jun- and JunD-dependent transcription. Effectively, these two proteins belong to the same family of transcription factors, but they are very different proteins. At the expression level, c-Jun is barely present in quiescent fibroblasts but its expression increases dramatically following serum stimulation [20], whereas JunD is expressed at significant levels in quiescent cells and slightly increases after induction of cell proliferation [21]. c-Jun is a positive regulator of Ras-mediated transformation, while JunD acts as a negative regulator of Ras-mediated transformation [22]. Moreover, c-Jun is a positive regulator of the cell cycle, as shown by multiple experiments including overexpression of c-Jun in fibroblasts [23] and studies of c-Jun-deficient mouse embryonic fibroblasts [24], while JunD acts as a negative regulator of the cell cycle as shown by overexpression in fibroblasts [23]. One of the reasons why c-Jun and JunD display opposite effects on cell cycle progression is that, while c-Jun is a strong activator of cyclin D1, JunD is a weak positive activator [25]. More generally, c-Jun, JunB and JunD behave differently on several of their target genes. For example, c-Jun and JunB stimulate p55CDC (cdc20) expression while JunD represses it, c-Jun and JunB stimulate the kinase PAK3 expression while JunD has almost no action on it, but c-Jun represses the Notch signaling pathway involved in protein HES-1 expression far more than JunB or JunD [26]. Since HBZ is encoded by the retrovirus HTLV-I, which is itself involved in cellular transformation, this opposite action of HBZ on AP-1 factors that have an opposite action on the cell cycle regulation and on transformation is of high significance.

Moreover, this finding that HBZ has an opposite action on c-Jun- and JunD-dependent transcription raises an interesting question concerning the role of HBZ on Tax-induced AP-1 transcription. As mentioned in Section 1, the viral protein Tax has multiple functions in the development of ATL, exerted through the dysregulation of the transcription of several viral and cellular genes including those encoding the AP-1 transcription factors. To investigate if HBZ had an effect on Tax-induced AP-1 transcription, CEM cells were cotransfected with Tax and HBZ as described above. As expected, Tax was found to activate the expression of the luciferase reporter gene by about 3.6-fold (Fig. 3D), and the luciferase activity was repressed in the presence of HBZ. This result supports the hypothesis of Basbous et al. that HBZ could be a negative modulator of the Tax effect by controlling Tax expression at the transcriptional level and by attenuating activation of AP-1 by Tax [13]. Moreover, given the different effects of HBZ on the three cellular Jun factors and given that HBZ represses Tax-mediated AP-1 transcription, we can postulate that Tax exerts its effect on AP-1 via c-Jun.

In conclusion, we can more generally postulate that HBZ is able to activate the transcription of various cellular genes which transcription is JunD-dependent. Further experiments will be required to understand which other genes can be activated by HBZ and why HBZ has an opposite effect on c-Jun- and JunD-dependent transcription.

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