



HAL
open science

The HBZ factor of human T-cell leukemia virus type I dimerizes with transcription factors JunB and c-Jun and modulates their transcriptional activity.

Jihane Basbous, Charlotte Arpin, Gilles Gaudray, Marc Piechaczyk, Christian Devaux, Jean-Michel Mesnard

► To cite this version:

Jihane Basbous, Charlotte Arpin, Gilles Gaudray, Marc Piechaczyk, Christian Devaux, et al.. The HBZ factor of human T-cell leukemia virus type I dimerizes with transcription factors JunB and c-Jun and modulates their transcriptional activity.. *Journal of Biological Chemistry*, 2003, 278 (44), pp.43620-7. 10.1074/jbc.M307275200 . hal-00188768

HAL Id: hal-00188768

<https://hal.science/hal-00188768>

Submitted on 27 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

The HBZ Factor of Human T-cell Leukemia Virus Type I Dimerizes with Transcription Factors JunB and c-Jun and Modulates Their Transcriptional Activity*

Received for publication, July 8, 2003, and in revised form, August 21, 2003
Published, JBC Papers in Press, August 22, 2003, DOI 10.1074/jbc.M307275200

Jihane Basbous^{‡§}, Charlotte Arpin[‡], Gilles Gaudray^{‡¶}, Marc Piechaczyk^{||}, Christian Devaux[‡], and Jean-Michel Mesnard^{‡**}

From the [‡]Laboratoire Infections Rétrovirales et Signalisation Cellulaire, CNRS/Université Montpellier I, Unité Mixte de Recherche 5121/Institut Fédératif de Recherche 122, Institut de Biologie, 4 Boulevard Henri IV, 34960 Montpellier Cedex 2, France and the ^{||}Institut de Génétique Moléculaire, UMR 5535/IFR 122, 1919 Route de Mende, 34293 Montpellier Cedex 5, France

The human T-cell leukemia virus type I (HTLV-I)-encoded Tax protein activates transcription from the viral promoter via association with the cellular basic leucine zipper factor cAMP-response element protein-2. Tax is also able to induce cellular transformation of T lymphocytes probably by modulating transcriptional activity of cellular factors, including nuclear factor- κ B, E2F, activator protein-1 (AP-1), and p53. Recently, we characterized in HTLV-I-infected cells the presence of a novel viral protein, HBZ, encoded by the complementary strand of the HTLV-I RNA genome (Gaudray, G., Gachon, F., Basbous, J., Biard-Piechaczyk, M., Devaux, C., and Mesnard, J.-M. (2002) *J. Virol.* 76, 12813–12822). HBZ is a nuclear basic leucine zipper protein that down-regulates Tax-dependent viral transcription by inhibiting the binding of cAMP-response element-binding protein-2 to the HTLV-I promoter. In searching for other cellular targets of HBZ, we identified two members of the Jun family, JunB and c-Jun. Co-immunoprecipitation and cellular colocalization confirmed that HBZ interacts *in vivo* with JunB and c-Jun. When transiently introduced into CEM cells with a reporter gene containing the AP-1 site from the collagenase promoter, HBZ suppressed transactivation by c-Jun. On the other hand, the combination of HBZ with Jun-B had higher transcriptional activity than JunB alone. Consistent with the structure of its basic domain, we demonstrate that HBZ decreases the DNA-binding activity of c-Jun and JunB. Last, we show that c-Jun is no longer capable of activating the basal expression of the HTLV-I promoter in the presence of HBZ *in vivo*. Our results support the hypothesis 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.

The activator protein-1 (AP-1)¹ transcription complex is involved in a multitude of cellular processes such as proliferation, differentiation, and cell death (1). Various AP-1 components, including c-Jun (2), can induce oncogenic transformation upon chronic activation in avian and mammalian cells (3, 4). The ability to regulate such a number of biological processes is due to the formation of various dimers between the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, and Fra-2) family members through their leucine zipper, a structural motif involving a heptad repeat of leucine residues. Jun proteins form both homodimers and heterodimers with Fos proteins (5–7), but Fos proteins are unable to homodimerize and require heterodimerization to bind to DNA (8–10). Dimers formed by Fos and Jun bind with the highest affinity to the AP-1 site, but they are also able to recognize the cAMP-response element (CRE) motif (11–13). The AP-1 complexes are not limited to Jun and Fos dimers because certain Jun and Fos proteins have been shown to dimerize with other basic leucine zipper (bZIP) proteins, including members of the activating transcription factor/CRE-binding protein (CREB) family and the Maf transcription factors generating complexes with altered binding specificities (4, 14, 15). The relative binding affinities of the AP-1 transcription factors depend on the specific DNA sequence, on the promoter context, and on the dimer combinations (16).

Human T-cell leukemia virus type I (HTLV-I) is an oncogenic retrovirus etiologically associated with the development of adult T-cell leukemia. HTLV-I transforms T-cells via its regulatory protein Tax (17), which interferes with cell growth control pathways through activation of cellular transcription factors, including nuclear factor- κ B (18), E2F (19), and AP-1 (20), and inactivation of p53 (21). Previous studies have shown that T-cell lines transformed by HTLV-I express high levels of AP-1 activity (20, 22) with increased levels of mRNAs coding for c-Jun, JunB, JunD, c-Fos, and Fra-1 (23, 24). It has been demonstrated that Tax induces expression of genes coding for c-Fos (25–27), Fra-1 (23, 28), c-Jun (23), and JunD (23). Moreover, AP-1 sites have been characterized as responsive elements for Tax in different cellular genes, including *fra-1* (28), interleukin-2 (29), interleukin-5 (30), interleukin-8 (31), *TR3/nur77* (32), and *TIMP-1* (33). It has been suggested that Tax could be involved in AP-1 regulation at a post-transcriptional

* This work was supported by grants from CNRS and the Université Montpellier I and by a grant from the Fondation de France and Association pour la Recherche sur le Cancer Grant 5933 (to J.-M. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Fellow of CNRS (Bourse Docteur Ingénieur).

¶ Fellow of the Ministère de l'Éducation Nationale, de la Recherche, et de la Technologie.

** To whom correspondence should be addressed: Lab. Infections Rétrovirales et Signalisation Cellulaire, Inst. de Biologie, 4 Blvd. Henri IV, CS 89508, 34960 Montpellier Cedex 2, France. Tel.: 33-4-6760-8660; Fax: 33-4-6760-4420; E-mail: jean-michel.mesnard@univ-montp1.fr.

¹ The abbreviations used are: AP-1, activator protein-1; CRE, cAMP-response element; bZIP, basic leucine zipper; CREB, cAMP-response element-binding protein; HTLV-I, human T-cell leukemia virus type I; HBZ, HTLV-I bZIP factor; GST, glutathione S-transferase; C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP-homologous protein.

level (20, 22), but the exact mechanism of activation remains misunderstood.

Tax is also involved in the transcription control of the HTLV-I genome itself (34). The elements that impart Tax responsiveness to the promoter consist of three 21-bp repeats containing an imperfect CRE sequence. Because Tax does not bind CRE by itself, previous studies have focused on identifying cellular proteins that bind to the viral CRE in conjunction with Tax. Using the yeast two-hybrid approach, the cellular bZIP transcription factor CREB-2 has been identified as a protein that interacts with Tax (35, 36). It has also been shown that Tax enhances CREB-2 binding to the 21-bp repeats (37), probably by promoting homodimerization of the CREB-2 bZIP domain (38, 39). Then, the promoter-bound Tax molecule recruits the transcriptional coactivator CREB-binding protein, which is involved in the acetylation of the core histone tails (40, 41). AP-1 is also able to transactivate the HTLV-I promoter *in vivo* (42, 43). However, no direct interactions between the AP-1 factors and Tax have been characterized, suggesting that AP-1 is obviously involved in basal transcription of the HTLV-I genome.

Recently, we characterized a novel HTLV-I protein encoded by the complementary strand of the viral RNA genome (44) and named by us HBZ for HTLV-I bZIP factor (45). The structure of HBZ resembles that of a prototypical bZIP transcription factor, with an N-terminal transcriptional activation domain and a C-terminal bZIP domain. HBZ acts as a repressor of viral transcription by forming heterodimers with CREB-2 that are no longer able to bind to the viral CRE (45). Here, through a combination of *in vitro* and *in vivo* experiments, we demonstrate that HBZ also interacts with two other bZIP factors, JunB and c-Jun. Our analysis reveals that HBZ decreases the DNA-binding activity of JunB and c-Jun, as is the case for CREB-2. In addition, we show that c-Jun is no longer capable of activating the basal expression of the HTLV-I promoter in the presence of HBZ *in vivo*. Our results support the hypothesis 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.

MATERIALS AND METHODS

Yeast Two-hybrid Screen—MT2 cDNA fused to the Gal4 activation domain of the pGAD10 vector (35) was screened using the HBZ bZIP domain as a bait fused to the Gal4 DNA-binding domain of the pGBT9 vector. The two-hybrid screen was performed with the *Saccharomyces cerevisiae* HF7c reporter strain (3×10^6 clones were screened) as already described (35).

Immunoprecipitation and Western Blotting—For immunoprecipitation of HBZ, plasmid pCI-HBZ, pcDNA-JunB, or pcDNA-c-Jun was cotransfected into 293T cells using the FuGENE 6 transfection reagent (Roche Applied Science) as recommended by the manufacturer. Cultures were grown to saturation in Dulbecco's modified Eagle's medium supplemented with 1% penicillin and streptomycin antibiotic mixture and 10% fetal calf serum. After centrifugation, cells were lysed in 50 mM Tris-HCl (pH 8), 100 mM NaCl, 1 mM EDTA, 1 mM MgCl₂, and 1% Triton X-100, and proteins were immunoprecipitated from protein extracts (2.5 mg of total proteins) by incubation at 4 °C for 2 h using rabbit anti-HBZ antiserum (45), followed by another incubation with protein A/G-agarose for 1 h. Bound fractions were washed six times and then electrophoresed on SDS-10% polyacrylamide gel and analyzed by immunoblotting as described (46). Mouse anti-JunB and anti-c-Jun antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and Oncogene Research Products (San Diego, CA), respectively.

Fluorescence Microscopy Analysis—COS-7 cells were transfected with 1 μg of plasmid pEGFP-HBZ, expressing a green fluorescent protein-HBZ fusion protein (45), and 1 μg of pcDNA-JunB or pcDNA-c-Jun. Cells were cultured on glass slides and then analyzed by fluorescence 24 h after transfection as described (35, 38). Jun proteins were detected using the above-cited mouse antibodies and secondary goat anti-mouse IgG antibody coupled to rhodamine (Pierce). Analysis

of the green, red, and merged fluorescence was carried out using a fluorescence microscope.

Transfections and Luciferase Assays—CEM cells were transiently cotransfected according to a previously published procedure (46). 5 μg of pcDNA3.1-lacZ (β-galactosidase-containing reference plasmid) was included in each transfection to control transfection efficiency. The total amount of DNA in each transfection was the same, the balance being made up with empty plasmids. Cell extracts equalized for protein content were used for luciferase and β-galactosidase assays. For the assays with the Gal4-binding site promoter-reporter plasmid, JunB or c-Jun fused in-frame with the DNA-binding domain of Gal4 into vector pBIND was cotransfected into CEM cells in the presence of the luciferase reporter plasmid pG5luc, containing five Gal4-binding sites upstream of a minimal TATA box.

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

Streptavidin-Biotin Complex Assay—A biotinylated oligonucleotide containing the AP-1 site (5'-cgcttgTAGAGTCAGcggaa-3') was annealed with its complementary oligonucleotide to form a double-stranded DNA. Biotinylated double-stranded DNA was incubated with bacterially produced proteins in 200 μl of binding buffer containing 50 mM Tris (pH 7.5), 500 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5 mM MgCl₂, 0.1% Triton, 5% glycerol, and 10 mg/ml bovine serum albumin at room temperature for 2 h before addition of streptavidin beads (Pierce). After a 1-h incubation at 4 °C, the beads were extensively washed with binding buffer without bovine serum albumin. The proteins that remained bound to the beads were eluted in SDS loading buffer and analyzed by Western blotting.

RESULTS

Binding of HBZ to JunB and c-Jun—To identify proteins that interact with HBZ, a two-hybrid approach in yeast was performed with the bZIP domain of HBZ (amino acids 123–209) as a bait. The yeast strain expressing the HBZ bZIP domain fused to the DNA-binding domain of Gal4 was transformed with a library of cDNA derived from the HTLV-I-infected MT2 cell line fused to the transcriptional activation domain of Gal4 (35). Several positive clones were found to correspond to two members of the Jun family, JunB and c-Jun (Fig. 1, A and B). The leucine zipper domain was, as expected, necessary for the interaction between both cellular factors and HBZ because truncated mutants of JunB (amino acids 1–273) and c-Jun (amino acids 1–287) lacking the leucine zipper did not bind to HBZ (Fig. 1C). To be sure of the specificity of the test in yeast, JunB and c-Jun were also tested in the presence of an unrelated protein, lamin, which was unable to interact with either of the two proteins (Fig. 1C). Moreover, HBZ was unable to form homodimers in yeast, as shown in Fig. 1C. To eliminate the possibility that the binding of HBZ to JunB or c-Jun may be indirect and dependent on yeast components, fusion proteins of either JunB or c-Jun with glutathione S-transferase (GST) were produced in *Escherichia coli*, and their binding to [³⁵S]methionine-labeled HBZ, produced in rabbit reticulocyte lysate, was analyzed. As shown in Fig. 2A, HBZ bound to JunB (lane 3) or c-Jun (lane 6) fused to GST, but not to GST alone (lanes 2 and 5). Taken together, our results show that HBZ interacts with JunB and c-Jun *in vitro*.

HBZ Co-immunoprecipitates with JunB and c-Jun *in Vivo*—To examine the *in vivo* interaction between HBZ and JunB, we coexpressed both proteins in 293T cells. Cell extracts were then immunoprecipitated with either rabbit anti-HBZ antiserum or preimmune serum from the same rabbit (45),

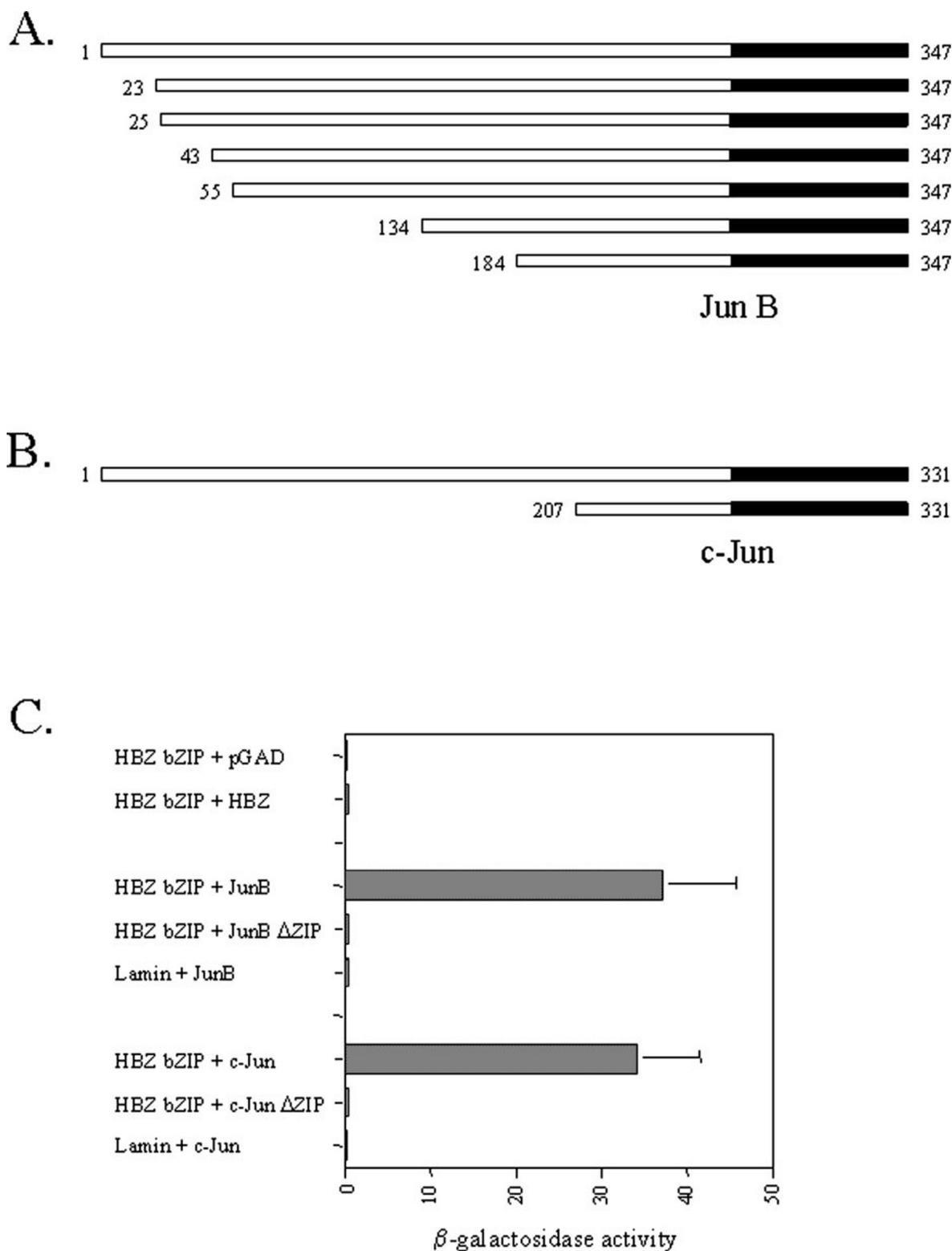


FIG. 1. Isolation by yeast two-hybrid screening of two members of the Jun family (JunB and c-Jun) that interact with the bZIP domain of HBZ. *A* and *B*, clones isolated corresponding to Jun B and c-Jun, respectively. The numbers shown indicate amino acid positions, and the bZIP domain is indicated by black boxes. *C*, analysis of the interactions between HBZ and JunB or c-Jun using a liquid culture β -galactosidase assay. Yeast cells were transformed with the expression vector pGAD, containing the entire coding sequence of JunB or c-Jun fused to the Gal4 activation domain, together with pGBT9, expressing the Gal4 DNA-binding domain fused to the HBZ bZIP domain or to lamin. pGBT-HBZ bZIP was also cotransformed with a plasmid expressing the Gal4 activation domain fused to full-length HBZ or to a truncated form of JunB or c-Jun without the leucine zipper (*JunB* Δ ZIP and *c-Jun* Δ ZIP, respectively). The β -galactosidase assay with *o*-nitrophenyl- β -D-galactopyranoside as substrate was carried out on three independent colonies per transformation as recommended by Clontech. The mean values (expressed in Miller units) are shown.

followed by Western analysis using anti-JunB monoclonal antibody. By this approach, JunB was found in the immunoprecipitate with anti-HBZ antiserum (Fig. 2*B*, lane 2), but not in

the control immunoprecipitate (lane 3). When the same experiment was performed with extracts of 293T cells transfected only with JunB, no protein was found in the immunoprecipitate

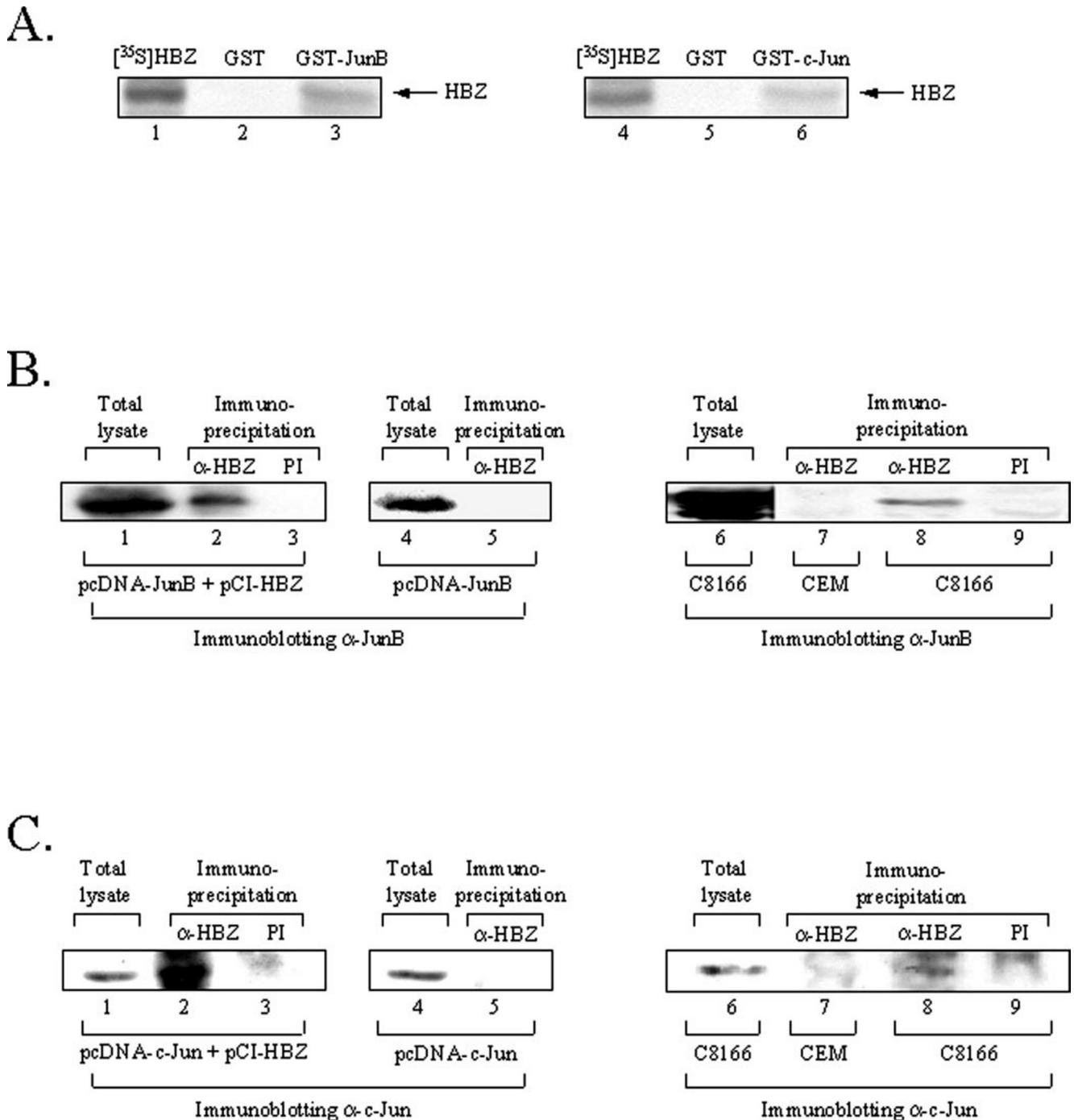


FIG. 2. HBZ interacts with JunB and c-Jun. *A*, *in vitro* binding assays carried out with HBZ and JunB or c-Jun. Equal amounts of GST (lane 2) and GST-JunB (lane 3) immobilized on glutathione-Sepharose beads were incubated with ^{35}S -labeled HBZ (lane 1), and bound proteins were analyzed by SDS-PAGE and autoradiography. The same assay was carried out with GST-c-Jun (lane 6). *B*, HBZ-JunB association *in vivo*. 293T cells were transfected with HBZ expression vector pCI-HBZ (lanes 1–3) or the corresponding empty vector (lanes 4 and 5) and plasmid pcDNA-JunB (lanes 1–5). Proteins from total lysates were directly probed with mouse anti-JunB antibody (lanes 1 and 4) or immunoprecipitated with rabbit anti-HBZ antibody (lanes 2 and 5) or preimmune serum (PI; lane 3), followed by Western analysis with anti-JunB antibody (lanes 2, 3, and 5). Proteins from the total lysate of the HTLV-I-infected C8166 cells (lanes 8 and 9) or uninfected CEM cells (lane 7) were also immunoprecipitated with anti-HBZ antibody (lane 7 and 8) or preimmune serum (lane 9), and immunoprecipitated proteins were analyzed with anti-JunB antibody (lanes 7–9). Lane 6 corresponds to C8166 proteins directly probed with anti-JunB antibody. *C*, HBZ-c-Jun association *in vivo*. The experiment was performed as described for HBZ and JunB, but with mouse anti-c-Jun antibody.

with anti-HBZ antiserum (Fig. 2*B*, lane 5), confirming the specificity of the interaction between HBZ and JunB. To confirm that both endogenous HBZ and JunB could also associate in infected cells, immunoprecipitation from a cell lysate of the HTLV-I-infected C8166 cell line expressing HBZ (45) was performed with anti-HBZ antiserum and probed with anti-JunB antibody. As expected, this approach revealed the presence of a complex between HBZ and JunB in C8166 extracts (Fig. 2*B*,

lane 8). By the same approach, such a complex was also detected in MT4 cells (data not shown), another HTLV-I-infected cell line, but not in the uninfected CEM cell line (Fig. 2*B*, lane 7). Altogether, these results clearly demonstrate that HBZ and JunB interact *in vivo*. As shown in Fig. 2*C*, the same co-immunoprecipitation experiments were carried out with c-Jun. The data obtained show that HBZ also bound to c-Jun *in vivo*.

HBZ Colocalizes with JunB and c-Jun in the Nucleus—The

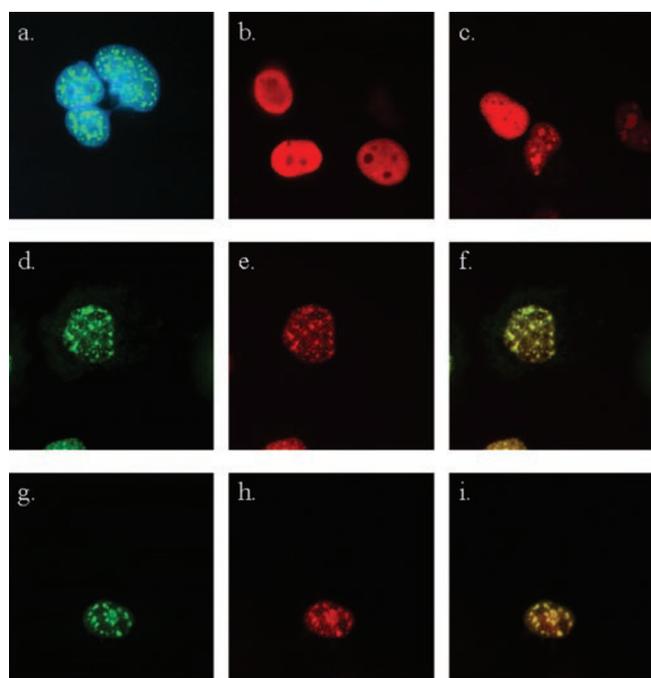


FIG. 3. Immunofluorescence microscopy analysis of the colocalization of HBZ and JunB or c-Jun *in vivo*. COS-7 cells were cotransfected with pEGFP-HBZ (a), pcDNA-JunB (b), pcDNA-c-Jun (c), pEGFP-HBZ + pcDNA-JunB (d-f), or pEGFP-HBZ + pcDNA-c-Jun (g-i). JunB (b and e) and c-Jun (c and h) were detected using mouse antisera and goat anti-mouse IgG antibodies coupled to rhodamine. Analysis of the green (d and g), red (e and h), and merged fluorescence (yellow; f and i) was performed with an immunofluorescence microscope. The blue fluorescence (a) of the nuclei (Hoechst staining) was visualized by UV illumination.

HBZ-JunB interaction was further investigated in COS-7 cells by confirming the co-distribution of the proteins by immunofluorescence microscopy. In transient transfection assays, we found that HBZ tagged with green fluorescent protein exhibited a granular distribution in the nucleus (Fig. 3a), as described previously (45). On the other hand, although JunB was also localized in the nucleus, here it showed a diffuse pattern (Fig. 3b). However, when JunB was cotransfected with HBZ, both proteins colocalized in the nuclear spots formed by the viral protein (Fig. 3, d-f). The same approach was also performed with c-Jun in COS-7 cells. Unlike JunB, c-Jun was distributed in either a diffuse or punctate staining pattern throughout the nucleus (Fig. 3c). Although we have already observed the existence of these two different staining patterns for other cellular proteins (47), we do not know the exact mechanisms of their formation. However, like JunB, after cotransfection with HBZ, c-Jun localized with HBZ in the nuclear spots (Fig. 3, g-i). These observations support the notion that HBZ and either JunB or c-Jun colocalize in the nucleus, but also show that HBZ entails an intranuclear redistribution of both JunB and c-Jun. Taken together with co-immunoprecipitation, our results strongly suggest that HBZ and either JunB or c-Jun can interact with each other *in vivo*.

HBZ Decreases the DNA-binding Activity of c-Jun—We next examined the effect of HBZ on transcription from the collagenase promoter containing a canonical AP-1 element. The reporter plasmid was first cotransfected into CEM cells with pcDNA-c-Jun in the presence of increasing amounts of the HBZ expression vector pCI-HBZ. As shown in Fig. 4A, c-Jun alone activated expression of the luciferase reporter gene by 34-fold; but this stimulation was inhibited in the presence of HBZ, and this inhibitory effect was proportional to the quantity of transfected HBZ plasmid. Moreover, we checked that the level of

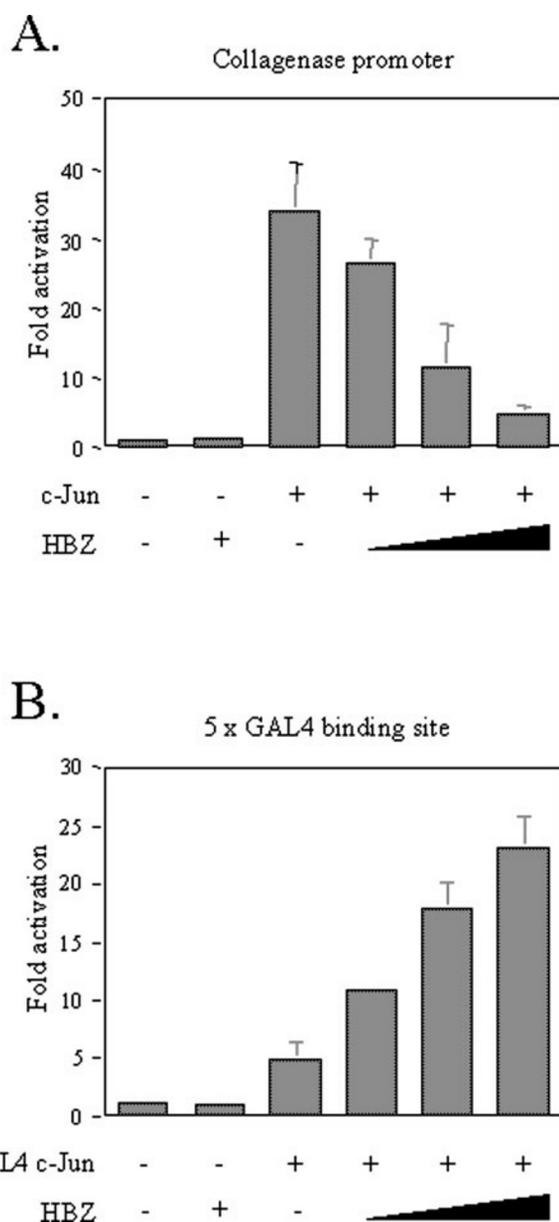


FIG. 4. HBZ inhibits transactivation by c-Jun. A, HBZ down-regulates c-Jun-dependent transcription. CEM cells were cotransfected with the collagenase promoter-driven luciferase gene (2 μ g), pcDNA3.1-lacZ (β -galactosidase-containing reference plasmid; 5 μ g), the c-Jun expression vector pcDNA-c-Jun (1 μ g), and pCI-HBZ (0, 1, 2, and 3 μ g). The luciferase values are expressed as increases relative to those of cells transfected with pcDNA and pCI-neo in the presence of the luciferase reporter gene. The total amount of DNA in each series of transfection was equal, the balance being made up with empty plasmids. Luciferase values were normalized for β -galactosidase activity. Values represent means \pm S.D. ($n = 3$). B, HBZ stimulates c-Jun activity when c-Jun is bound to the promoter. CEM cells were cotransfected with the luciferase reporter vector pG5luc (1 μ g), pBIND (expressing Gal4-c-Jun; 1 μ g), and increasing amounts of pCI-HBZ (0, 1, 2, and 3 μ g). The luciferase values were normalized for β -galactosidase activity and are expressed as -fold increase relative to that of cells transfected with pG5luc, pBIND, and pCI-Neo. Values represent means \pm S.D. ($n = 3$).

c-Jun expressed from pcDNA-c-Jun was not significantly decreased by the expression of HBZ (data not shown). Thus, HBZ negatively modulates c-Jun activity on a promoter containing the AP-1 element.

Two simple mechanisms may explain the repression of c-Jun activity by HBZ. First, HBZ and c-Jun may form heterodimers that may not bind to the AP-1 site. If the heterodimer does not bind to the AP-1 motif, obviously it cannot stimulate transcrip-

tion. Second, for unknown reasons, the complex between HBZ and c-Jun bound to the AP-1 site is much less active than the c-Jun homodimers. To discriminate between these two possibilities, we fused the full-length c-Jun protein to the DNA-binding domain of the yeast transcription factor Gal4 and used this chimera to analyze the effect of HBZ on c-Jun directly bound to a promoter bearing Gal4-binding sites. The Gal4-c-Jun chimera was assayed using the reporter plasmid pG5luc, encoding luciferase under the control of five Gal4-binding sites upstream of a minimal TATA box. After cotransfection of CEM cells with pG5luc and Gal4-c-Jun, luciferase expression was stimulated by 5-fold (Fig. 4B). When increasing amounts of HBZ were added, luciferase expression was stimulated by 23-fold (Fig. 4B). These results show that HBZ is no longer capable of inhibiting c-Jun activity when c-Jun is stably bound to the promoter. Consequently, they also suggest that HBZ could negatively regulate c-Jun activity by forming heterodimers that are unable to form stable complexes with the AP-1 site.

To confirm this hypothesis, we compared c-Jun DNA-binding activity in the presence and absence of HBZ. The first series of experiments were based on the analysis of c-Jun DNA-binding activity by the microwell colorimetric assay from Active Motif Europe (48). Nuclear cell extracts of 293T cells transfected with c-Jun alone or associated with HBZ or c-Fos were incubated in the presence of a double-stranded oligonucleotide containing the AP-1 site (TGAGTCA) immobilized in microwell plates. c-Jun specifically bound to this oligonucleotide was then detected using anti-c-Jun antiserum. As shown in Fig. 5A, c-Jun bound to the AP-1 oligonucleotide, and this binding was stimulated by c-Fos; on the other hand, HBZ decreased c-Jun binding. In the second series, we used the streptavidin-biotin complex assay as previously described to analyze the inhibitory effect of HBZ on the DNA-binding activity of CREB-2 (45). Bacterially purified GST-c-Jun was incubated with a double-stranded oligonucleotide containing the AP-1 site in the absence or presence of the bZIP domain of HBZ, also purified from bacteria (45). c-Jun alone bound to the AP-1 oligonucleotide (Fig. 5B, lane 2); but in the presence of the HBZ bZIP domain, the c-Jun interaction with AP-1 was abolished (lanes 4 and 5). Taken together, our results demonstrate that HBZ prevents c-Jun from binding to the AP-1 site. On the other hand, as shown in Fig. 5C, when c-Jun was pre-bound to the AP-1 probe, HBZ was unable to displace c-Jun.

HBZ Modulates JunB Activity—We also analyzed the effect of HBZ on JunB-mediated transcriptional stimulation of the collagenase promoter in CEM cells. As expected, JunB alone activated transcription of the reporter luciferase gene (Fig. 6A); but as already shown by others (49, 50), this activation was very weak compared with c-Jun (Fig. 4A). Indeed, JunB has been described as a poorly efficient activator of the collagenase promoter and can inhibit activation of the promoter by c-Jun (49, 50). Interestingly, the combination of HBZ with JunB showed a transcriptional activity slightly higher than with JunB alone (~3-fold), but this effect was not dose-dependent (Fig. 6A). We next examined the effect of HBZ on JunB DNA-binding activity. JunB homodimers are known to have no or very weak DNA binding *in vitro* (13). For this reason, using the microwell colorimetric assay, we analyzed JunB DNA-binding activity from nuclear cell extracts of 293T cells transfected with JunB alone or associated with HBZ or c-Fos, as described above for c-Jun. As shown in Fig. 6B, HBZ attenuated JunB binding to the AP-1 site. As already observed for c-Jun, these results demonstrate that HBZ forms heterodimers with JunB that cannot stably bind to DNA.

Transactivation of the HTLV-I Promoter by c-Jun Is Inhibited in the Presence of HBZ—c-Jun is involved in the basal

expression of the HTLV-I promoter (42, 43), with the viral CRE sites being the functional c-Jun-responsive targets in the promoter (42). In light of our results, HBZ should be able to down-regulate viral transcription in the presence of c-Jun. For this reason, HeLa cells were cotransfected with a luciferase reporter construct carrying the HTLV-I promoter and increasing amounts of pCI-HBZ in the presence of c-Jun. As shown in Fig. 7, c-Jun alone activated expression of the luciferase reporter gene by ~10-fold, but this stimulation was inhibited in the presence of HBZ (only 1.5-fold). Thus, HBZ is capable of inhibiting HTLV-I basal transcription.

DISCUSSION

We have previously shown that HBZ forms heterodimers with CREB-2 that are no longer able to bind to the viral CRE sequence (45). In this study, we have demonstrated that HBZ is also able to interact *in vitro* and *in vivo* with two other bZIP transcription factors that belong to the Jun family, c-Jun and JunB. As described for CREB-2 and the CRE motif, HBZ also decreases the binding of c-Jun and JunB to the AP-1 site and then controls their transcriptional activity. This negative effect of HBZ can be explained by important substitutions of residues that are generally conserved in the DNA-binding domains of other bZIP factors. The presence of alanine residues substituting for conserved amino acids in the basic region of HBZ (45) may play a determining role in preventing the attainment of the structural conformation necessary for binding to the CRE and AP-1 sites.

The role of HBZ is unlikely to be restricted to that of an inhibitor because HBZ contains a potential activation domain located in its N terminus (45). Indeed, although HBZ decreases the DNA-binding activity of JunB, the association of the two proteins gives rise to a weak but significant transcriptional activity from the collagenase promoter in CEM cells. This result can be explained by the particular properties of JunB compared with those of c-Jun. It was found that despite their similar primary structure and DNA-binding specificities, they differ greatly in their ability to activate transcription of AP-1-dependent promoters (13, 51). Whereas c-Jun is an effective activator of the human collagenase promoter, JunB is rather ineffective. This difference is caused by a small number of amino acid substitutions between the JunB bZIP domain and the corresponding c-Jun sequence. These changes lead to a 10-fold decrease in the DNA-binding activity of JunB (13, 51). In addition, two glycine residues present in the JunB leucine zipper decrease its stability, resulting in decreased homodimerization and increased heterodimerization (51). In view of these observations, we can assume that the HBZ-JunB combination has a very weak DNA-binding activity, but probably forms a more stable dimer than the JunB homodimer. The formation of such a complex could explain the observed activating effect of HBZ on JunB. From now on, understanding the physiological significance of these interactions and the molecular mechanisms by which HBZ modulates JunB and c-Jun activities is a major goal for further research.

It is interesting to note that HBZ presents some analogies to the cellular protein CHOP (C/EBP homologous protein). The latter dimerizes with CREB-2 (38), activating transcription factor-3 (52), and members of the CCAAT/enhancer-binding protein (C/EBP) family (53). The basic region of CHOP deviates considerably in sequence from that of other bZIP proteins, and the heterodimers formed are unable to bind to the typical CRE and C/EBP sites (52, 53). However, CHOP is also able to bind as a heterodimer with C/EBP α and C/EBP β to specific DNA control elements and then to positively regulate gene expression (54, 55). From these observations, it would be of interest to determine whether HBZ forms heterodimers with c-Jun and

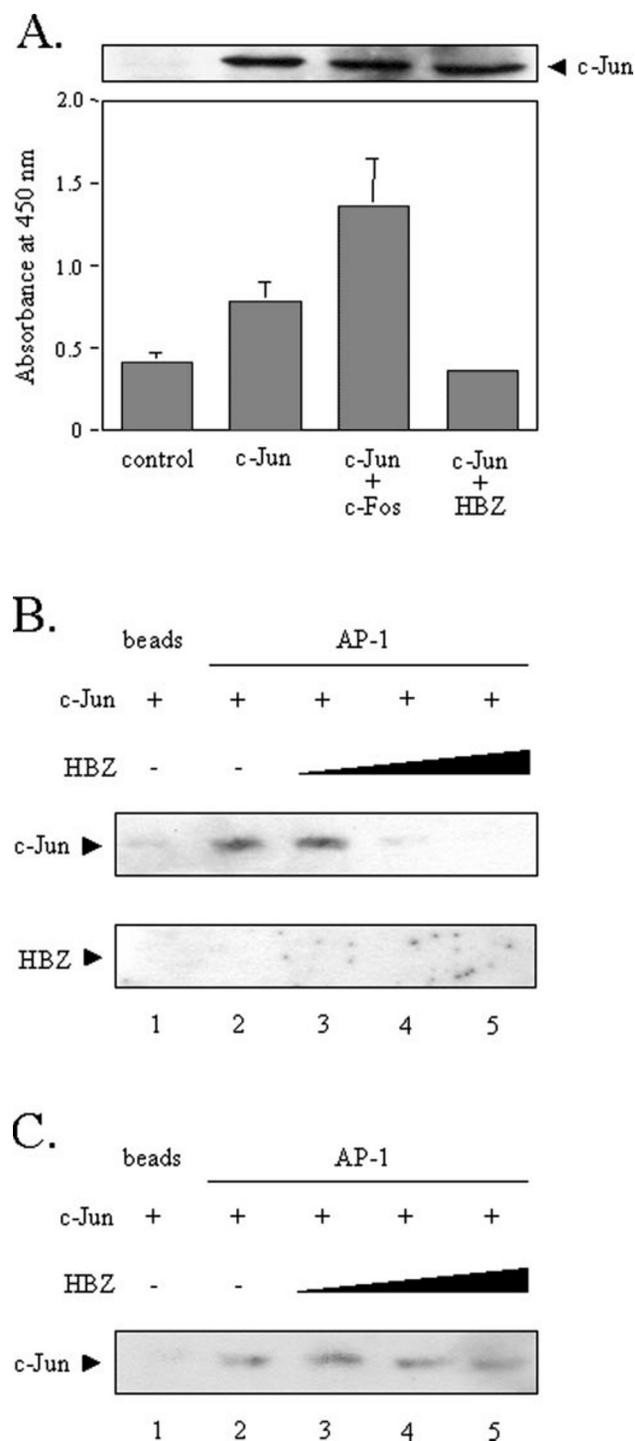


FIG. 5. HBZ decreases the DNA-binding activity of c-Jun. *A*, microwells containing the DNA probe with the AP-1 site were incubated with nuclear cell extracts of 293T cells transfected with c-Jun alone (3 μ g of pcDNA-c-Jun) or associated with HBZ (3 μ g of pCI-HBZ) or c-Fos (3 μ g of c-Fos expression vector pcDNA-c-Fos). The negative control corresponds to cells transfected with plasmid pcDNA. The data represent means \pm S.D. ($n = 3$). An immunoblot of nuclear proteins from transfected 293T cells using anti-c-Jun antibody is shown. *B*, HBZ abolishes the binding of c-Jun to the AP-1 site. A double-stranded oligonucleotide (100 ng) containing the AP-1 site was incubated with 25 ng of c-Jun in the absence (*lane 2*) or presence (*lanes 3–5*) of the bZIP domain of HBZ (5, 25, and 50 ng, respectively). The complexes were collected on streptavidin beads, and the proteins bound to the beads were analyzed by Western blotting with anti-c-Jun antiserum (*upper panel*) or anti-HBZ antiserum (*lower panel*). *C*, HBZ does not displace c-Jun pre-bound to the AP-1 site. The AP-1 probe was first incubated with 25 ng of c-Jun (*lane 2*) for 1 h and then with 5, 25, and 50 ng of the bZIP domain of HBZ (*lanes 3–5*, respectively) for one additional hour. The proteins bound to the AP-1 site were analyzed as described for *B*.

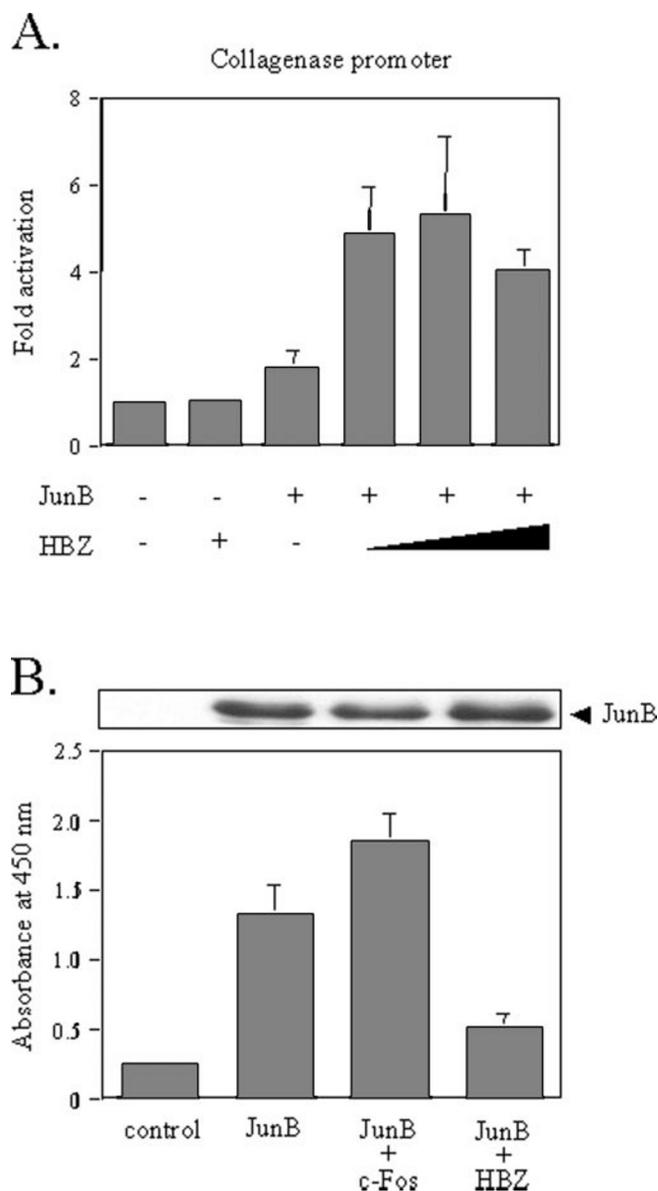


FIG. 6. HBZ modulates the transcriptional activity of JunB. *A*, HBZ activates JunB-dependent transcription. CEM cells were cotransfected as described in the legend to Fig. 4, but with pcDNA-JunB instead of pcDNA-c-Jun. Values represent means \pm S.D. ($n = 3$). *B*, microwells containing the DNA probe with the AP-1 site were incubated with nuclear cell extracts of 293T cells transfected with JunB alone (3 μ g of pcDNA-c-Jun) or associated with HBZ (3 μ g of pCI-HBZ) or c-Fos (3 μ g of pcDNA-c-Fos). The negative control corresponds to cells transfected with plasmid pcDNA. The data represent means \pm S.D. ($n = 3$).

JunB capable of binding to specific sequences different from the AP-1 and CRE motifs.

We have also demonstrated here that HBZ down-regulates Jun-dependent transcription and therefore decreases basal viral transcription by interacting with c-Jun. Moreover, we have already shown that HBZ inhibits Tax-dependent viral transcription by interacting with CREB-2 (45). Thus, HBZ seems capable of counteracting the effect of Tax by acting at two different levels. Not only can HBZ control Tax production at the transcriptional level, but it also can modulate Tax activity in the infected cells by attenuating activation of AP-1. It is not surprising that the HTLV-I genome can encode such a protein because Tax is a strong activator, and its uncontrolled overexpression can lead to cell death. Modulation of Tax activity in a timely fashion may be critical for maintaining the viability of the host cell to allow the effective replication of HTLV-I. Other

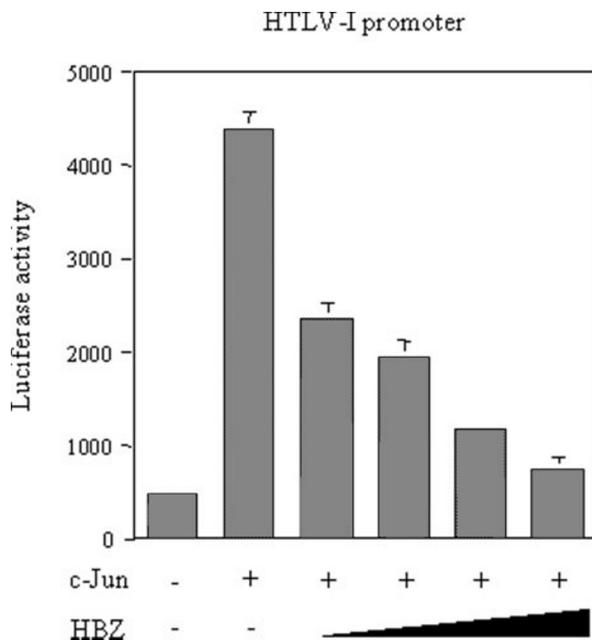


FIG. 7. HBZ down-regulates HTLV-1 basal transcription controlled by c-Jun. HeLa cells were cotransfected with a luciferase reporter construct containing the HTLV-1 long terminal repeat (1 μ g), pcDNA-c-Jun (5 μ g), and pCI-HBZ (0, 1, 2, 3, and 4 μ g). Values represent means \pm S.D. ($n = 3$).

oncoviruses are known to encode similar proteins that buffer activity of strong activators. Thus, Kaposi's sarcoma-associated herpesvirus codes for a bZIP protein (K-bZIP) that modulates the function of K-Rta, a transcription factor involved in activation of viral transcription (56, 57). Another interesting example is the papilloma virus E2 protein. Human papilloma virus expresses the E6 and E7 oncoproteins, which are sufficient to immortalize primary human keratinocytes. Like Tax (58), E6 and E7 interfere with cell cycle control by inactivating p53 (59) and pRb (60), respectively. On the other hand, E2 represses the viral promoter directing expression of E6 and E7, with the loss of E2 expression contributing to carcinogenesis (61). Indeed, human papilloma virus DNA is generally found integrated in cancers, and integration often results in the disruption of the E2 gene and loss of its expression. Interestingly, HBZ is poorly expressed in cell lines derived from leukemic cells (45). Therefore, it will be of great interest to determine whether the inhibition of HBZ expression is a necessary step for T-cell transformation by HTLV-I.

REFERENCES

- Shaulian, E., and Karin, M. (2002) *Nat. Cell Biol.* **4**, 131–136
- Vogt, P. K. (2001) *Oncogene* **20**, 2365–2377
- Mechta-Grigoriou, F., Feral, D., and Yaniv, M. (2001) *Oncogene* **20**, 2378–2389
- van Dam, H., and Castellazzi, M. (2001) *Oncogene* **20**, 2453–2464
- Gentz, R., Rauscher, F. J., Abate, C., and Curran, T. (1989) *Science* **243**, 1695–1699
- Kouzarides, T., and Ziff, E. (1988) *Nature* **15**, 646–651
- Ransone, L. J., Visvader, J., Sassone-Corsi, P., and Verma, I. M. (1989) *Genes Dev.* **6**, 770–781
- Cohen, D. R., and Curran, T. (1990) *Oncogene* **5**, 929–939
- Nakabeppu, Y., and Nathans, D. (1989) *EMBO J.* **8**, 3833–3841
- Ransone, L. J., Wamsley, P., Morley, K. L., and Verma, I. M. (1990) *Mol. Cell. Biol.* **10**, 4565–4573
- Hai, T., and Curran, T. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 3720–3724
- Nakabeppu, Y., Ryder, K., and Nathans, D. (1988) *Cell* **55**, 907–915
- Ryseck, R.-P., and Bravo, R. (1991) *Oncogene* **6**, 533–542

- Kerppola, T. K., and Curran, T. (1994) *Oncogene* **9**, 675–684
- Macgregor, P. F., Abate, C., and Curran, T. (1990) *Oncogene* **5**, 451–458
- Chinenov, Y., and Kerppola, T. K. (2001) *Oncogene* **20**, 2438–2452
- Basbous, J., Gaudray, G., Devaux, C., and Mesnard, J.-M. (2002) *Recent Res. Dev. Mol. Cell. Biol.* **3**, 155–166
- Xiao, G., Cvijic, M. E., Harhaj, E. W., Yuhlik, M. T., Waterfield, M., and Sun, S.-C. (2001) *EMBO J.* **20**, 6805–6815
- Lemasson, I., Thébault, S., Sardet, C., Devaux, C., and Mesnard, J.-M. (1998) *J. Biol. Chem.* **273**, 23598–23604
- Iwai, K., Mori, N., Oie, M., Yamamoto, N., and Fujii, M. (2001) *Virology* **279**, 38–46
- Suzuki, T., Uchida-Toita, M., and Yoshida, M. (1999) *Oncogene* **18**, 4137–4143
- Mori, N., Fujii, M., Iwai, K., Ikeda, S., Yamasaki, Y., Hata, T., Yamada, Y., Tanaka, Y., Tomonaga, M., and Yamamoto, N. (2000) *Blood* **95**, 3915–3921
- Fujii, M., Niki, T., Mori, T., Matsuda, T., Matsui, M., Nomura, N., and Seiki, M. (1991) *Oncogene* **6**, 1023–1029
- Hooper, W. C., Rudolph, D. L., Lairmore, M. D., and Lal, R. B. (1991) *Biochem. Biophys. Res. Commun.* **181**, 976–980
- Alexandre, C., and Verrier, B. (1991) *Oncogene* **6**, 543–551
- Fujii, M., Sassone-Corsi, P., and Verma, I. M. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 8526–8530
- Nagata, K., Ohtani, K., Nakamura, M., and Sugamura, K. (1989) *J. Virol.* **63**, 3220–3226
- Tsuchiya, H., Fujii, M., Niki, T., Tokuhara, M., Matsui, M., and Seiki, M. (1993) *J. Virol.* **67**, 7001–7007
- Curtiss, V. E., Smilde, R., and McGuire, K. L. (1996) *Mol. Cell. Biol.* **16**, 3567–3575
- Yamagata, T., Mitani, K., Ueno, H., Kanda, Y., Yazaki, Y., and Hirai, H. (1997) *Mol. Cell. Biol.* **17**, 4272–4281
- Mori, N., Mukaida, N., Ballard, D. W., Matsushima, K., and Yamamoto, N. (1998) *Cancer Res.* **58**, 3993–4000
- Liu, X., Chen, X., Zachar, V., Chang, C., and Ebbesen, P. (1999) *J. Gen. Virol.* **80**, 3073–3081
- Uchijima, M., Sato, H., Fujii, M., and Seiki, M. (1994) *J. Biol. Chem.* **269**, 14946–14950
- Thébault, S., Gachon, F., Gaudray, G., and Mesnard, J.-M. (2001) *Recent Res. Dev. Virol.* **3**, 151–164
- Gachon, F., Péleraux, A., Thébault, S., Dick, J., Lemasson, I., Devaux, C., and Mesnard, J.-M. (1998) *J. Virol.* **72**, 8332–8337
- Reddy, T. R., Tang, H., Li, X., and Wong-Staal, F. (1997) *Oncogene* **14**, 2785–2792
- Gachon, F., Thébault, S., Peleraux, A., Devaux, C., and Mesnard, J.-M. (2000) *Mol. Cell. Biol.* **20**, 3470–3481
- Gachon, F., Gaudray, G., Thébault, S., Basbous, J., Koffi, A. J., Devaux, C., and Mesnard, J.-M. (2001) *FEBS Lett.* **502**, 57–62
- Wagner, S., and Green, M. R. (1993) *Science* **262**, 395–399
- Gachon, F., Devaux, C., and Mesnard, J.-M. (2002) *Virology* **299**, 271–278
- Georges, S. A., Kraus, W. L., Luger, K., Nyborg, J. K., and Laybourn, P. J. (2002) *Mol. Cell. Biol.* **22**, 127–137
- Jiang, K.-T., Chiu, R., Santos, E., and Kim, S.-J. (1991) *Virology* **181**, 218–227
- Lemasson, I., Polakowski, N. J., Laybourn, P. J., and Nyborg, J. K. (2002) *J. Biol. Chem.* **277**, 49459–49465
- Larocca, D., Chao, L. A., Seto, M. H., and Brunck, T. K. (1989) *Biochem. Biophys. Res. Commun.* **163**, 1006–1013
- Gaudray, G., Gachon, F., Basbous, J., Biard-Piechaczyk, M., Devaux, C., and Mesnard, J.-M. (2002) *J. Virol.* **76**, 12813–12822
- Thébault, S., Gachon, F., Lemasson, I., Devaux, C., and Mesnard, J.-M. (2000) *J. Biol. Chem.* **275**, 4848–4857
- Thébault, S., Basbous, J., Gay, B., Devaux, C., and Mesnard, J.-M. (2000) *Eur. J. Cell Biol.* **79**, 834–838
- Renard, P., Ernest, I., Houbion, A., Art, M., Le Calvez, H., Raes, M., and Remacle, J. (2001) *Nucleic Acids Res.* **29**, E21
- Chiu, R., Angel, P., and Karin, M. (1989) *Cell* **59**, 979–986
- Schütte, J., Viallet, J., Nau, M., Segal, S., Fadorok, J., and Minna, J. (1989) *Cell* **59**, 987–997
- Deng, T., and Karin, M. (1993) *Genes Dev.* **7**, 479–490
- Chen, B. P. C., Wolfgang, C. D., and Hai, T. (1996) *Mol. Cell. Biol.* **16**, 1157–1168
- Ron, D., and Habener, J. F. (1992) *Genes Dev.* **6**, 439–453
- Sok, J., Wang, X.-Z., Batchvarova, N., Kuroda, M., Harding, H., and Ron, D. (1999) *Mol. Cell. Biol.* **19**, 495–504
- Ubeda, M., Wang, X.-Z., Zinszner, H., Wu, I., Habener, J. F., and Ron, D. (1996) *Mol. Cell. Biol.* **16**, 1479–1489
- Izumiya, Y., Lin, S.-F., Ellison, T., Chen, L.-Y., Izumiya, C., Luciw, P., and Kung, H.-J. (2003) *J. Virol.* **77**, 1441–1451
- Liao, W., Tang, Y., Lin, S.-F., Kung, H.-J., and Giam, C. Z. (2003) *J. Virol.* **77**, 3809–3815
- Mesnard, J.-M., and Devaux, C. (1999) *Virology* **257**, 277–284
- Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M. (1990) *Cell* **63**, 1129–1136
- Dyson, N., Howley, P. M., Munger, K., and Harlow, E. (1989) *Science* **243**, 934–937
- Wells, S. I., Francis, D. A., Karpova, A. Y., Dowhanick, D. A., Benson, J. D., and Howley, P. M. (2000) *EMBO J.* **19**, 5762–5771