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HTLV-1 Basic Leucine-Zipper Factor, HBZ, Interacts With MafB and Suppresses Transcription Through a Maf Recognition Element

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ABSTRACT

HTLV-1 infection causes adult T-cell leukemia (ATL). The development of ATL is thought to be associated with disruption of transcriptional control of cellular genes. HTLV-1 basic leucine-zipper (bZIP) factor, HBZ, is encoded by the complementary strand of the provirus. We previously reported that HBZ interacts with c-Jun and suppresses its transcriptional activity. To identify the cellular factor(s) that interact with HBZ, we conducted a yeast two-hybrid screen using full-length HBZ as bait and identified MafB. HBZ heterodimerizes with MafB via each bZIP domain. Luciferase analysis revealed a significant decrease in transcription through Maf recognition element (MARE) in a manner dependent on the bZIP domain of HBZ. Indeed, production of full-length HBZ in cells decreased the MARE-bound MafB protein, indicating that HBZ abrogates the DNA-binding activity of MafB. In addition, HBZ reduced the steady-state levels of MafB, and the levels were restored by treatment with a proteasome inhibitor. These results suggest a suppressive effect of HBZ on Maf function, which may have a significant role in HTLV-1 related pathogenesis. J. Cell. Biochem. 111: 187–194, 2010.

KEY WORDS: HTLV-1; HBZ; LARGE MAF; TRANSCRIPTIONAL REGULATION; b-ZIP FACTOR

Human T-cell leukemia virus type-1 (HTLV-1) infection causes adult T-cell leukemia (ATL) in 2–5% of carriers after a long latent period. HTLV-1 is also closely related to HTLV-1-associated myelopathy or tropical spastic paraparesis (HAM/TSP) [Uchiyama, 1997]. Disruption of transcriptional control of cellular genes by viral proteins is thought to be associated with the development of the malignant ATL phenotype.

The viral Tax protein, encoded by the *env-pX* gene [Seiki et al., 1983], plays a principal role in the regulation of the proliferation and transformation of HTLV-1-infected T cells. Tax is a pleiotropic factor that interacts with many cellular proteins to regulate gene expression, including Bcl-xL, interleukin-2 (IL-2), and IL-2 receptor, in order to regulate proliferation and transformation of HTLV-1-infected cells [Yamaoka et al., 1992; Akagi and Shimotohno, 1993]. Moreover, Tax also directly binds to and suppresses the function of $p16^{ink4a}$, a member of the INK4 family of CDK inhibitors [Suzuki et al., 1996; Iwanaga et al., 2001; Haller et al., 2002], which leads to aberrant cell cycle progression. These findings highlight the oncogenic properties of Tax. Interestingly, ATL cells often contain genetic and epigenetic alternations such as deletion of 5′-long terminal repeat (LTR) or hypermethylation, resulting in the loss of Tax expression [Matsuoka, 2005]. This paradox between the oncogenic properties of Tax and silencing of Tax expression in ATL remains to be explained.

Abbreviations used: ATL, adult T-cell leukemia; bZIP, basic region-leucine zipper; HBZ, HTLV-1 bZIP factor; HTLV-1, human T-cell leukemia virus type 1; MARE, Maf-recognition element; HA, hemagglutinin; PCR, polymerase chain reaction.T. Ohshima and R. Mukai contributed equally to this work.

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In addition to Tax, other viral accessory proteins have been shown to regulate HTLV-1 transcription, one of which is HTLV-1 bZIP factor (HBZ). This protein is encoded in the complementary strand of the provirus and transcribed from a promoter located within the 3′-LTR [Larocca et al., 1989; Cavanagh et al., 2006; Murata et al., 2006]. Both the spliced (HBZ-SI) and unspliced (HBZ) transcripts encode highly related protein isoforms that have been detected most ATL cells [Larocca et al., 1989; Murata et al., 2006; Satou et al., 2006]. HBZ isoforms are nuclear proteins that contain a transactivation domain and a conserved bZIP domain in its N- and C-termini, respectively. HBZ interacts with the AP-1 family of transcription factors, c-Jun [Basbous et al., 2003; Matsumoto et al., 2005], JunB [Basbous et al., 2003], JunD [Thébault et al., 2004] through its bZIP domain. We have recently demonstrated that HBZ interacts with c-Jun and not only impairs the DNA-binding activity of c-Jun, but also promotes its proteasomal degradation [Matsumoto et al., 2005]. Although most proteasomal substrates are targeted for degradation via conjugation of polyubiquitin chains, however, we have reported that ubiquitination is not required for HBZ-mediated proteasomal degradation of c-Jun [Isono et al., 2008]. HBZ acts as a tethering factor between the proteasome and its substrate, thereby mimicking the targeting function of ubiquitination.

In this study, we searched for cellular factors that interacted with HBZ by yeast two-hybrid screening system. This approach identified a member of the large Maf family of transcription factor, MafB,*** that associated with HBZ. Large Maf family share similar basic region/leucine zipper motifs that mediate DNA binding to Maf recognition elements (MARE) and dimer formation [Kataoka et al., 1994a,b]. HBZ interacted with MafB via the bZIP domain and inhibited activation of a gene containing MARE on the promoter in a manner dependent on bZIP-domain of HBZ. Further analysis of the functional regulation of MafB by HBZ revealed that HBZ not only suppresses the DNA-binding activity of MafB in MARE but also reduces its stability via a proteasome-dependent pathway.

MATERIALS AND METHODS

CELL CULTURE, TRANSFECTIONS, AND LUCIFERASE REPORTER ASSAY

HEK-293T and HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. These cells were maintained at 37°C in 5% CO_2 atmosphere. Transfections were performed using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. Luciferase activity was normalized to *Renilla* luciferase activity derived from co-transfected pRL-tk-Luc (Promega). All reporter assays were performed in triplicate, and the error bars in the figures denote the standard errors (SE).

ANTIBODIES AND REAGENTS

Mouse anti-HA (M132-3; MBL), mouse anti-Myc (9E10; Santa Cruz), rat anti-HA (3F10; Roche Diagnostics), mouse (M2) and rabbit anti-Flag (Sigma), horseradish peroxidase (HRP)-linked goat anti-rat IgG (Jackson ImmunoReseach Lab) were purchased. TrueBlot HRP-linked goat anti-mouse and anti-rabbit IgG antibodies were purchased from eBioscience. Cycloheximide and MG132 were purchased from Wako Pure Chemicals.

PLASMID CONSTRUCTION

Expression vectors for full length and HBZ deletion mutants were generated by inserting the *Eco*RI/*Xho*I fragments of pcDNA3-Flag-HBZs [Matsumoto et al., 2005; Isono et al., 2008] into the *Eco*RI/*Xho*I sites of pcDNA3 with 5×HA epitope tagged at the N-terminus. Full length and MafB deletion mutants were generated by PCR amplification using pEF-Flag-MafB [Kajihara et al., 2003] as templates, respectively. These fragments were subcloned into the pcDNA3 with 3×Flag and 4×Myc epitope tagged at the N-terminus. Primer sequences used were as follows: MafB full length, 5′-AAA**GAATTC**ATGGCCGCGGAGCTGAGCATG-3′ (forward), 5′-AAA**CTCGAG**TCACAGAAAGAACTCAGGAGAGG-3′ (reverse); MafB-N, 5′-AAA**GAATTC**ATGGCCGCGGAGCTGAGCATG-3′ (forward), 5′-AAA**CTCGAG**TCAGTGATGGTGCGGGTGAGCGTG-3′ (reverse); MafB-C, 5′-AAA**GAATTA**CATCATCACCAAGCGTCGCCC-3′ (forward), 5′-AAA**CTCGAG**TCACAGAAAGAACTCAGGAGAGG-3′ (reverse); MafB-ΔLZ, 5′-AAA**GAATTC**ATGGCCGCGGAGCTGAGCATG-3′ (forward), 5′-AAA**CTCGAG**TCAGTGATGTTTCTGCTGGACGCG-3′ (reverse). Bold letters in the primers denote restriction sites. pαCE2-Luc, a luciferase reporter plasmid containing six copies of the MARE site, has been described previously [Kajihara et al., 2003].

YEAST TWO-HYBRID SCREENING

The full-length DNA fragment of HBZ in pACT2 (Clontech) was used as a bait to screen a human spleen cDNA library according to the manufacturer's instructions (Clontech). The transformants were plated onto selective medium lacking histidine, tryptophan, and leucine, and containing 150 mM 3-aminotriazole (3-AT) to isolate clones with histidine prototropy. Selected clones were assayed for another marker, the β-galactosidase activity, as described in the Clontech protocol. Plasmid DNA was extracted from positive clones and analyzed by partial DNA sequencing.

IMMUNOPRECIPITATIONS

HEK-293T cells (1×10^6 per 6 cm-diameter dish) were transfected with expression plasmids using Lipofectamine 2000. At 36 h post-transfection, cells were lysed in 1 ml of lysis buffer (25 mM Tris–HCl [pH 8.0], 100 mM NaCl, 0.1% NP-40, 1 mM EDTA, and a complete protease inhibitor cocktail tablet [Roche Diagnostics]). Cell debris was removed by centrifugation for 15 min. Lysates were incubated with antibodies for 1 h at 4°C. Finally, the antibody complexes were captured with protein G-sepharose beads for 1 h. Beads were washed four times with the same buffer and immunoprecipitants were eluted and analyzed by Western blot.

IMMUNOFLUORESCENCE

HeLa cells were seeded on an 8-well chamber slide (7×10^3 cells/well) and transfected with expression plasmids using Lipofectamine 2000. At 5 h post-transfection, Toxi-Blocker transfection supplement (TOYOBO), which prevents the cytotoxicity of lipofection reagents, was added in culture cells. After an additional 36 h of incubation, cells were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS), and permeabilized in 0.1% Triton X-100 in

PBS at room temperature for 5 min. After blocking with 5% skim milk in PBS for 1 h, cells were incubated with mouse anti-Flag and rat anti-HA antibodies at room temperature for 1 h. Samples were washed four times and then incubated with Alexa 488-conjugated goat anti-mouse, 568-conjugated goat anti-rat antibodies (Molecular Probes), and 1 μg/ml of Hoechst33342 (Wako Pure Chemicals) at room temperature for 30 min. The cells were washed, mounted in 90% glycerol in PBS containing 0.01% *p*-phenylenediamine, and then visualized using an immnofluorescense microscope (OLYMPUS).

DNA AFFINITY PRECIPITATION ASSAY

HEK-293T cells (1×10^6 per 6 cm-diameter dish) were transfected with the appropriate expression plasmids. At 24 h post-transfection, 20 μM of MG132 was added to the cultures. After 15 h incubation, cells were lysed in 500 μl of binding buffer (25 mM Tris–HCl [pH 8.0], 100 mM NaCl, 0.5% NP-40, 1 mM EDTA, and a complete protease inhibitor cocktail tablet [Roche Diagnostics]). Cell debris was removed by centrifugation for 15 min. Lysates were incubated with 10 μg/ml poly(dI–dC; Sigma) and 2 μg of biotinylated oligonucleotide for 1 h at 4°C. Bound proteins were captured with Streptavidin–sepharose beads (Sigma) for 1 h. After washing the beads four times with the binding buffer, precipitated proteins were eluted and analyzed by western blot. 5′-biotinylated oligonucleotides including the MARE site (5′-CGCTTGATGACTCAGCCGGAA-3′) or the non-specific sequence (5′-AATTCCCGCGAGGGGCGCCTA-3′) were annealed with its complementary oligonucleotide.

PULSE-CHASE EXPERIMENT

HEK-293T cells were seeded on 6-well plates (3×10^5 cells/well) and transfected with the appropriate expression plasmids. After 24 h, cells were treated with 50 μM of cycloheximide and were chased for the indicated intervals. Harvested cells were resolved by SDS–PAGE and analyzed by western blot. Band intensities were measured using a LAS 3000 image analyzer (Fuji film).

RESULTS

IDENTIFICATION OF MafB AS A CELLULAR FACTOR THAT INTERACTS WITH HBZ

Previous studies from our group and others have described that HBZ interacts with the AP-1 family of transcription factors, including c-Jun, JunB, JunD through its bZIP domain and modulates AP-1-mediated transcription [Basbous et al., 2003; Thébault et al., 2004; Matsumoto et al., 2005]. We searched for other factor(s) that interacts with HBZ by yeast two-hybrid screen. Some positive clones were found to correspond to bZIP transcription factors, including c-Jun, JunB, CREB-2, and a number of clones encoded a protein of MafB, which is a member of the large Maf family.

To analyze the physical interaction of HBZ with MafB in mammalian cells, co-immunoprecipitation experiments were conducted using extracts from HEK-293T cells which co-produced HA-HBZ or HBZ-SI with Flag-MafB. Immunoprecipitants with anti-Flag antibody were resolved by SDS–PAGE and western blot using anti-HA antibody was conducted. The result showed that the bands reactive to the HA antibody were corresponding to the HBZ and HBZ-SI, respectively (Fig. 1). To investigate whether the endogenous interaction between HBZ and MafB was detected, we then generated of anti-HBZ antibody and conducted co-immunoprecipitation experiment using extracts from HTLV-1-infected cell lines (MT-1, -4, and HUT102). However, the specific interaction of HBZ with MafB was hardly detectable (data not shown). This inability to detect HBZ could be explained by a low level of HBZ protein in these cell lines, in agreement with previous reports [Gaudray et al., 2002; Suemori et al., 2009]. To clarify this issue, a more detailed study using high affinity and low background antibody is needed.

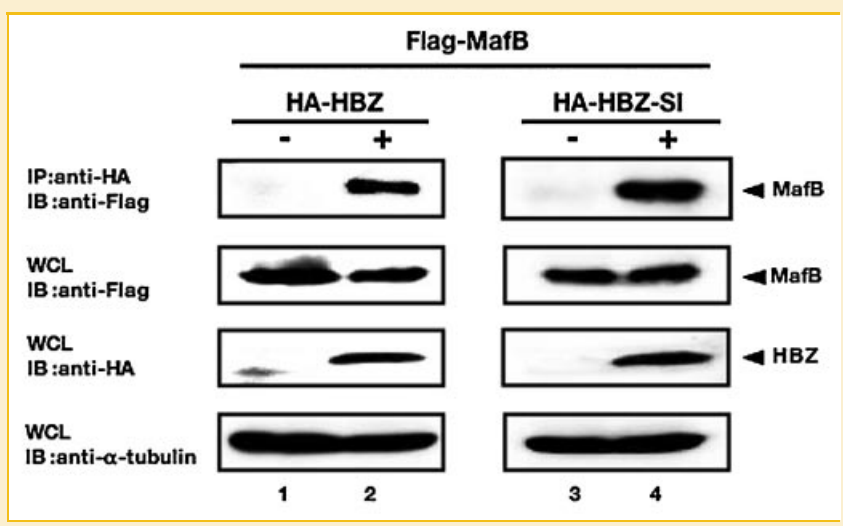

Fig. 1. MafB interacts with HBZ and HBZ-SI. HEK-293T cells were co-transfected with 3 μg of each plasmid expressing Flag-MafB together with (+) or without (−) 3 μg of plasmid expressing HA-HBZ or HBZ-SI. After 36 h, cell extracts were prepared and subjected to immunoprecipitation (IP) using anti-HA antibody followed by anti-Flag immunoblot (IB). Levels of protein in whole cell lysates (WCL) were analyzed by immunoblot using anti-Flag, anti-HA, or anti-α-tubulin antibodies.

DETERMINATION OF THE INTERACTION DOMAINS BETWEEN HBZ AND MafB

To define the domain within HBZ and MafB responsible for the interaction, we constructed deletion mutants (Fig. 2A,C) and performed immunoprecipitation assays. As shown in Figure 2B, MafB could be detected in the immunocomplexes isolated from cells lysates producing HBZ full length (HBZ-full) and HBZ-C-terminus (HBZ-C), but not HBZ-N-terminus (HBZ-N) and HBZ-ΔLZ (lacking leucine-zipper region). Likewise, MafB full length (MafB-full) and MafB-C-terminus (MafB-C) could interact with HBZ, but not MafB-N-terminus (MafB-N) and MafB-ΔLZ (lacking leucine-zipper region; Fig. 2D). Taken together, these results indicated that HBZ and Maf proteins form heterodimers via interactions in their bZIP domains.

SUBCELLULAR DISTRIBUTION OF HBZ AND MafB

The molecular interaction of HBZ with MafB may alter their mutual subcellular localization. Thus, we analyzed the cellular localization of HBZ and MafB in transiently transfected HeLa cells. Full-length HBZ alone localized to the nucleus in a speckled pattern (Fig. 3A), in agreement with our previous report [Matsumoto et al., 2005]. However, when HBZ and MafB were co-expressed in cells, HBZ no longer formed speckles but was diffusely distributed throughout the nucleus corresponding to the pattern of MafB alone (Fig. 3B, upper panels). In contrast, MafB-ΔLZ that was unable to associate with HBZ, did not affect HBZ localization (Fig. 3B, lower panels), suggesting that a physical interaction with MafB alters the distribution of HBZ.

HBZ SUPPRESSES TRANSCRIPTION THROUGH THE MARE SITES AND ABROGATES THE DNA-BINDING ACTIVITY OF MafB

Large Maf proteins share conserved bZIP domains that form dimers and bind DNA at MARE sites [Kataoka et al., 1994a]. An acidic region of Maf mediates transcriptional activation, and plays a key role in cellular differentiation [Nishizawa et al., 1989; Swaroop et al., 1992; Kataoka et al., 1994a; Ogino and Yasuda, 1998]. Next, we investigated the effect of HBZ on MafB-mediated transcription from a luciferase reporter containing MARE sites. HEK-293T cells were transfected with various combinations of plasmids expressing HBZ-SI, full-length HBZ, HBZ-N, HBZ-C, HBZ-ΔLZ, and MafB together with pαCE2-Luc. Exogenous expression of MafB stimulated luciferase activity by 13-fold relative to the basal level, and co-expression of HBZ-SI and full-length HBZ repressed this activity in a dose-dependent manner (Fig. 4A, lanes 3–6). However, the suppressive effect of HBZ-C was much lower than that of full-length

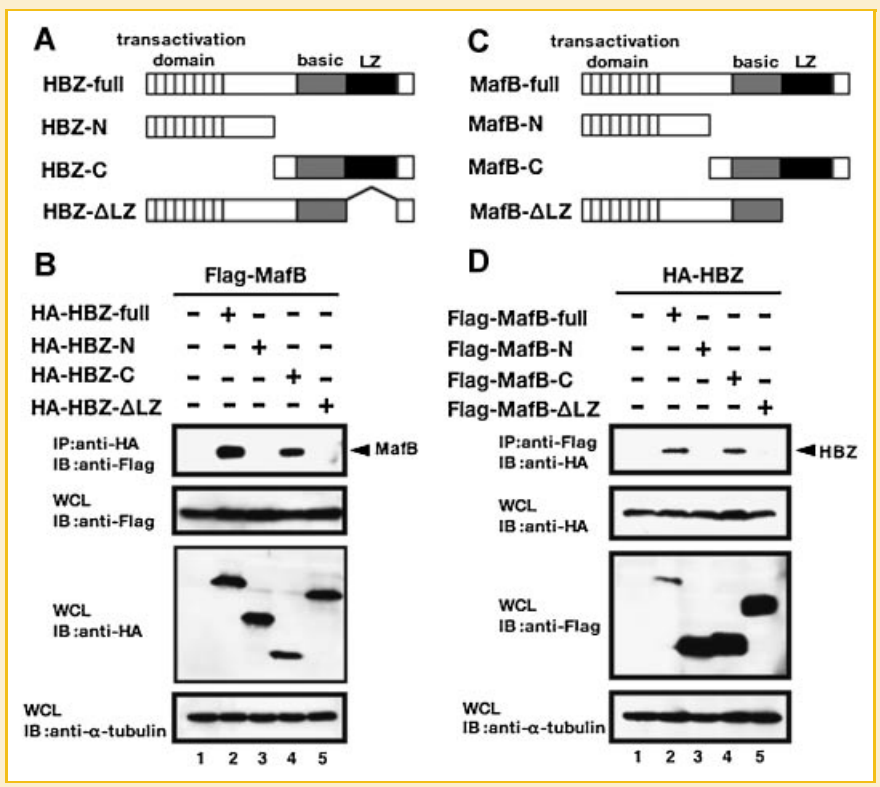

Fig. 2. HBZ and MafB formed heterodimers via their bZIP domains. A: Schematic diagram of HBZ and the mutants used in this study. Characteristic domains of HBZ are indicated as follows: transactivation domain, hatch; basic region, gray; and leucine-zipper (LZ) region, black. B: HEK-293T cells were co-transfected with 3 μg of plasmid expressing Flag-MafB full length (Flag-MafB-full) together with (+) or without (−) 3 μg of plasmid expressing HA-HBZ full length (HA-HBZ-full), -N, -C, or -ΔLZ. After 36 h, cell extracts were prepared and subjected to immunoprecipitation using anti-HA antibody followed by anti-Flag immunoblot. Levels of protein in whole cell lysates were analyzed by immunoblot. C: Schematic diagram of MafB and the mutants used in this study. Characteristic domains of MafB are indicated as follows: transactivation domain, hatch; basic region, gray; and LZ region, black. D: HEK-293T cells were co-transfected with 3 μg of plasmid expressing HA-HBZ full length together with (+) or without (−) 3 mg of plasmid expressing Flag-MafB full length, -N, -C, or -ΔLZ. After 36 h, cell extracts were prepared and subjected to immunoprecipitation using anti-Flag antibody followed by anti-HA immunoblot. Levels of protein in whole cell lysates are analyzed by immunoblot.

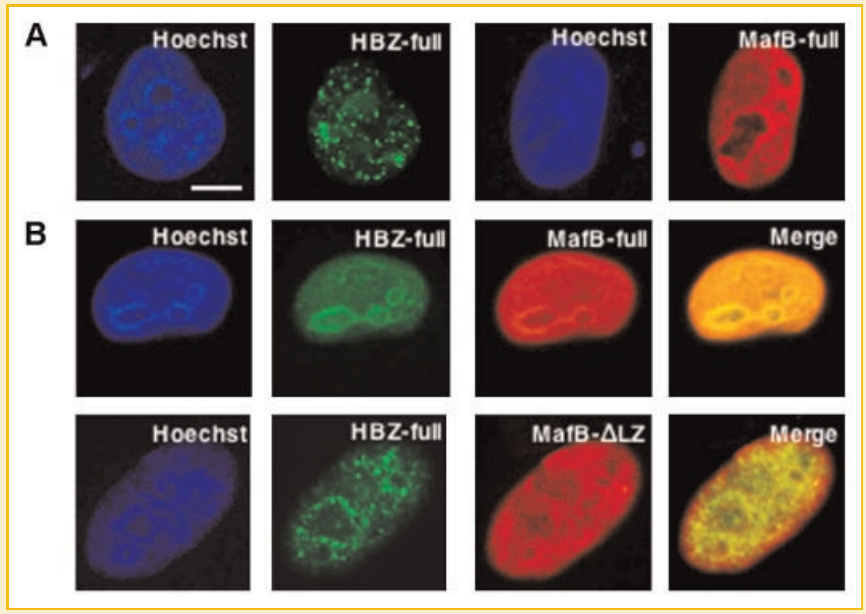

Fig. 3. Cellular localization of HBZ and MafB. A: HeLa cells were transfected with 1 μg of indicated plasmid expressing HA-HBZ full length (HBZ-full), or Flag-MafB full length (MafB-full) alone. After 5 h, the cells were treated with 1% toxi-blocker for 36 h. Cells expressing HA-HBZ-full (green) or Flag-MafB-full (red) were detected using anti-Flag or anti-HA antibodies. Hoechst 33342 (Hoechst) was used as a counterstain for nuclei. The bar represents 10 μm. B: HeLa cells were co-transfected with 1 μg of the indicated plasmids expressing HA-HBZ full together with Flag-MafB full, or HA-HBZ full together with Flag-MafB-ΔLZ. Cells expressing HA-HBZ-full (green) and Flag-MafB-full, or -ΔLZ (red) for the same field of cells were visualized using anti-Flag or -HA antibodies.

HBZ (lanes 5 and 6 vs. 9 and 10). In contrast, HBZ-N and HBZ-ΔLZ did not significantly inhibit transcription (lanes 7 and 8, 11 and 12). These data indicate that the LZ region of HBZ is required for its suppressive function, and full-length HBZ possess an additional function to fully suppress the transcriptional activity of MafB.

We have recently demonstrated that HBZ not only impairs the DNA-binding activity of c-Jun, but also enhances its proteasomal degradation [Matsumoto et al., 2005; Isono et al., 2008]. Thus, we investigated whether HBZ abrogates the DNA-binding activity of MafB. HEK-293T cells were transfected with empty vector, HBZ, HBZ-C, or HBZ-ΔLZ expression vectors together with the MafB expression vector. Cell lysates were incubated with a 5′-biotinylated DNA including the MARE sequence or a non-specific sequence, and protein-bound DNA were recovered with streptavidin-coated beads. As shown in Figure 4B, the amount of MafB bound to the MARE sequences decreased in cells that expressed full-length HBZ, but not HBZ-C and -ΔLZ. Unexpectedly, however, the expression of HBZ-C in cells led to an increase in the amount of MafB bound to the MARE (Fig. 4B, top panel), and that the band of HBZ-C was detected on MARE element (Fig. 4B, second panel). Since HBZ-C is not a naturally formed but an artificially truncated protein. Therefore, it is assumed that although HBZ-C can interact with MafB, it did not inhibit the DNA binding of MafB as the full-length HBZ.

HBZ PROMOTES MafB DEGRADATION THROUGH A PROTEASOME-DEPENDENT PATHWAY

Next, we examined whether HBZ accelerates the degradation of MafB via a proteasome-dependent pathway. Expression of HBZ markedly reduced the steady-state level of MafB protein (Fig. 5A, top panel, lanes 1 and 2), but expression of HBZ-C or HBZ-ΔLZ did not have such an effect (lanes 3 and 4). In contrast, cells treated with MG132, a proteasome inhibitor, revealed that the level of MafB was not significantly different between the lanes (5 and 8). This result further supports the notion that HBZ suppresses MafB protein level via a proteasome-dependent pathway.

Finally, we hypothesized that HBZ might reduce the steady-state levels of MafB protein through accelerating its degradation. To test this possibility, we performed a pulse-chase analysis of MafB in HEK-293T cells expressing MafB together with HBZ full length, HBZ-C or HBZ-ΔLZ exogenously. As shown in Figure 5B, in the presence of full-length HBZ, MafB proteins declined more rapidly than in cells expressed HBZ-C or -ΔLZ. This result indicated that HBZ reduced the steady-state levels of MafB protein by enhancing the turnover of MafB protein and the association with full-length HBZ is essential for degradation of the target proteins.

DISCUSSION

In this study, we have identified MafB, which is a member of large Maf transcription factors, as a cellular factor that interacted with HBZ using the yeast two-hybrid system. Interactions between HBZs consisting of non-spliced (HBZ) and spliced (HBZ-SI) isoforms and MafB transcription factor was confirmed in mammalian cells (Fig. 1). The HBZ isoforms differ by a few amino acids at the N-terminus of each protein. The domains in HBZ are highly conserved domain, with the N-terminus domain being involved in transcriptional activation, a central region that mediates nuclear localization, and the C-terminal domain containing bZIP [Hivin et al., 2006]. HBZ was associated with MafB through their ZIP domains (Fig. 2). We and

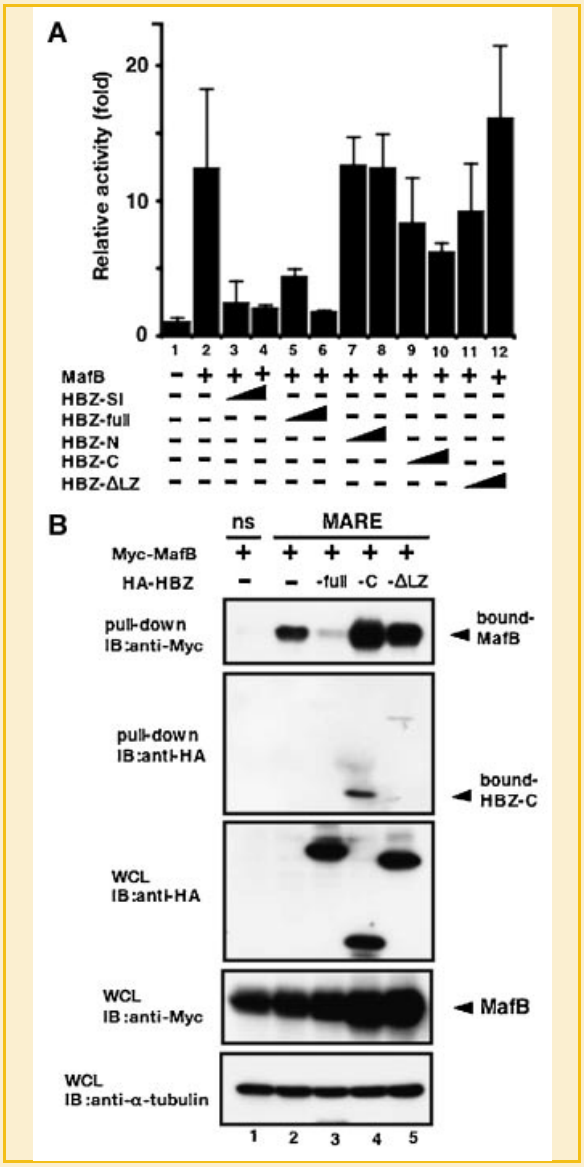

Fig. 4. The leucine-zipper region of HBZ mediates its suppressive function through the MARE-dependent transcription. A: HEK-293T cells were co-transfected with 25 ng of pαCE2-Luc reporter plasmid, 10 ng of pRL-tk-Luc, and 5 ng of pEF-Flag-MafB, or an empty plasmid (−) together with increasing amounts (100 or 200 ng) of pcDNA3-Flag-HBZ-SI, or -HBZ full length (HBZ-full), or -HBZ-N, or -HBZ-C, or -HBZ-ΔLZ, respectively. The total amount of DNA used for transfection was equalized by adding an empty plasmid. After 36 h, the luciferase activities were measured. The activity of the reporter plasmid alone in control cells was arbitrarily given a value of 1, and the activities of the other transfectants were adjusted relative to this assay. Each value represents the mean of a triplicate set of experiments ± SE. B: HBZ full length inhibits the DNA-binding ability of MafB. HEK-293T cells were transfected with 0.5 μg of pcDNA3-Myc-MafB together with (+) or without (−) 2.5 μg of pcDNA3-HA-HBZ full length, or -HBZ-C or -HBZ-ΔLZ. After 24 h, cells were treated with 20 μM of MG132 for 15 h. These cell lysates were subjected to the DNA affinity precipitation assay. 5′-Biotinylated oligonucleotides containing a non-specific sequence (ns) or a MARE sequence (MARE) were used. Pull-down complexes were eluted and analyzed by immunoblot using anti-Myc or anti-HA antibodies. Levels of protein in whole cell lysates (WCL) were analyzed by immunoblot using anti-HA, anti-Myc, or anti-α-tubulin antibodies.

other groups have previously reported that HBZ interacts with various cellular bZIP proteins, including ATF-4, JunB, JunD, and c-Jun, and modulates their transcriptional activity. Our observations using reporter gene assays demonstrated that full-length HBZ strikingly repressed the *trans*-acting function of MafB, but HBZ-ΔLZ caused a complete loss of its repressive activity (Fig. 4A). Surprisingly, although HBZ-C partially suppressed the transcriptional activity of MafB, the co-expression of MafB and HBZ-C, but not full-length HBZ, in cells resulted in an increased amount of MafB bound to the MARE (Fig. 4B, top panel), and that the HBZ-C also bound to the MARE-site DNA (Fig. 4B, second panel). The reason for the discrepancy in the results between the reporter assay and DNA precipitation experiment remain to be elucidated. It has been reported that heterodimer formation of Fos with small Maf proteins, which do not contain a canonical transactivation domain, are defective in *trans*-activation [Kataoka et al., 1995]. It is also well known that heterodimerization of the full length CCAATT/enhancer-binding protein β (C/EBPβ) with its truncated isoform, which possesses only bZIP domain, attenuates transcriptional activity [Lekstrom-Himes and Xanthopoulos, 1998]. Further, allosteric regulation in nuclear receptors heterodimer is needed as a molecular switch allowing either transcriptional activation or repression [Bain et al., 2007; Venäläinen et al., 2009]. In view of these reports, we hypothesized that there is an allosteric communication within the MafB/HBZ-C heterodimer and HBZ-C appears to act as a dominant negative form of HBZ protein. During review of this manuscript, a paper showed that HBZ can form a heterocomplex with MafB and MafG in vitro, and this association is dependent on the basic region of HBZ [Reinke et al., 2010]. Indeed, the marked suppression of transcription by full-length HBZ was the result of the impairment of, at least, two functions: the DNA-binding activity and the stability of MafB (Figs. 4 and 5). Based on our previously studies, the effect of HBZ on the regulation of Maf function is likely related to facilitate the delivery of MafB to the proteasome [Matsumoto et al., 2005; Isono et al., 2008].

The *v-maf* oncogene was originally identified in an avian transforming virus [Nishizawa et al., 1989]. Large Maf proteins encompass c-Maf, MafB, Nrl, and L-Maf and contain a bZIP domain and an acidic transactivation domain. Previous studies indicated the overproduction of c-Maf or MafB induced transformation of fibroblast cells [Kataoka et al., 1993, 1994a]. Moreover, Morito et al. have reported that c-Maf contributes to T-cell lymphoma in vivo in c-Maf transgenic mouse models [Morito et al., 2006]. Taken together, it seems that large Maf proteins might contribute to the development of cellular transformation. Since AP-1 has been implicated in the tumorigenesis and transformation of T-cells, inappropriate activation of AP-1 could contribute to the dysregulated phenotypes of HTLV-1-infected cells or the development of ATL. Indeed, HTLV-1-infected cell lines have high AP-1 activity with elevated levels of mRNAs encoding AP-1 components (c-Fos, JunB, JunD, c-Fos and Fra-1, etc.) [Fujii et al., 1991; Hooper et al., 1991]. The physiological function of HBZ remains to be elucidated. Previously, studies reported that HBZ is likely to be expressed in HTLV-1 infected cell lines and ATL patient-derived cells, and promotes $CD4^+$ T-lymphocyte proliferation [Satou et al., 2006]. Moreover, HBZ enhances infectivity and persistence in HTLV-1-

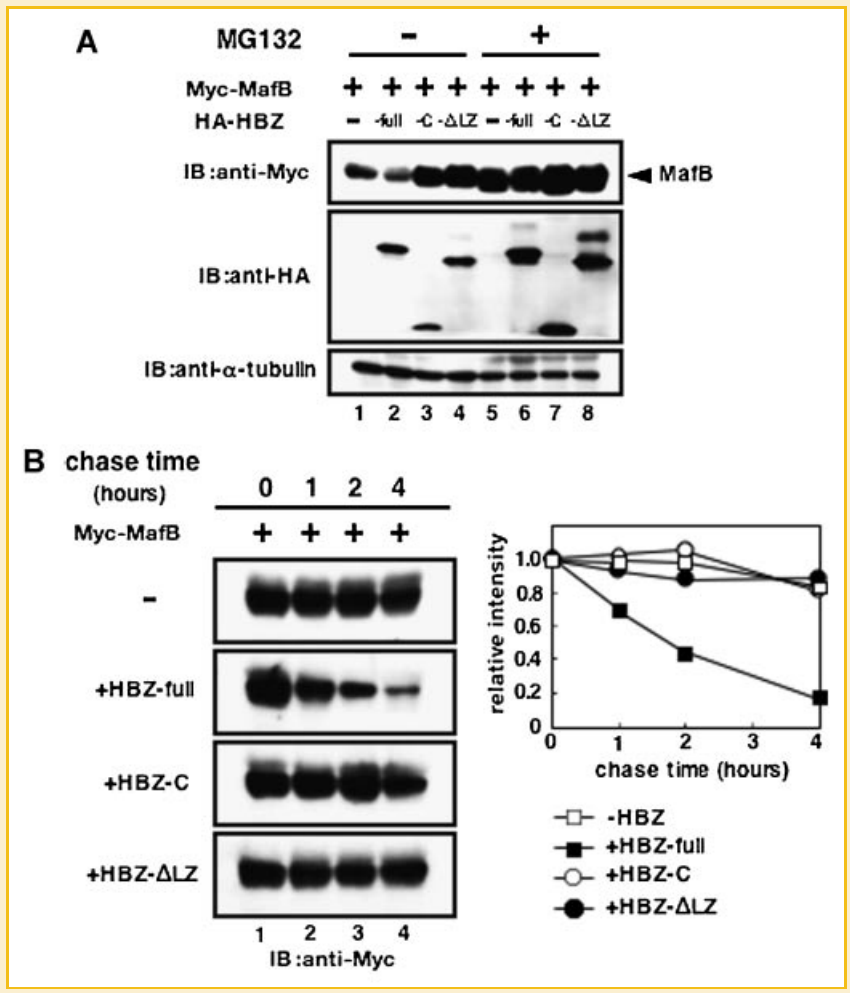

Fig. 5. HBZ suppresses the stability of MafB protein. A: HBZ promotes MafB degradation through a proteasome-dependent pathway. HEK-293T cells were co-transfected with 1 μg of plasmid expressing Myc-MafB together with (+) or without (−) 3 μg of plasmid expressing HA-HBZ full length (HBZ-full), or -HBZ-C, or -HBZ-ΔLZ. After 24 h, cells were treated with (lanes 5–8) or without (lanes 1–4) 20 μM MG132 (a proteasome inhibitor) for 16 h. The cell lysates were then subjected to SDS-PAGE, followed by immunoblot with anti-Myc, anti-HA, or anti-α-tubulin antibodies. B: HBZ facilitates MafB degradation. HEK-293T cells were transfected with 1 μg of plasmid expressing Myc-MafB together with (+) or without (−) 3 μg of plasmid expressing HA-HBZ full length, or, -C or -ΔLZ. After 24 h, the cells were treated with 50 μM cycloheximide and collected at the indicated times. Cell lysates were analyzed by immunoblot using anti-Myc antibody. The intensity of each band is quantified and graphed.

inoculated rabbits [Arnold et al., 2006]. Interestingly, HBZ also seems to be expressed at low levels in HTLV-1-infected cell lines [Gaudray et al., 2002] and it exhibits a significant suppressive effect of many tumorigenesis factors that contain a bZIP domain. This discrepancy will be of great interest, as we seek to define whether the inhibition of HBZ expression is involved in the development step or in the smoldering, chronic, and acute stages after a long latency of HTLV-1 infection. Further studies will be needed to define the role of HBZ in the development of T-cell transformation by HTLV-1. Clarification of the molecular mechanisms by which HBZ contributes to tumorigenesis may ultimately facilitate the discovery of a therapeutic agent for ATL.

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