The Sp1-like Protein BTEB3 Inhibits Transcription via the Basic Transcription Element Box by Interacting with mSin3A and HDAC-1 Co-repressors and Competing with Sp1*

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Sp1-like proteins are characterized by three conserved C-terminal zinc finger motifs that bind GC-rich sequences found in promoters of numerous genes essential for mammalian cell homeostasis. These proteins behave as transcriptional activators or repressors. Although significant information has been reported on the molecular mechanisms by which Sp1-like activators function, relatively little is known about mechanisms for repressor proteins. Here we report the functional characterization of BTEB3, a ubiquitously expressed Sp1-like transcriptional repressor. GAL4 assays show that the N terminus of BTEB3 contains regions that can act as direct repressor domains. Immunoprecipitation assays reveal that BTEB3 interacts with the co-repressor mSin3A and the histone deacetylase protein HDAC-1. Gel shift assays demonstrate that BTEB3 specifically binds the BTE site, a well characterized GC-rich DNA element, with an affinity similar to that of Sp1. Reporter and gel shift assays in Chinese hamster ovary cells show that BTEB3 can also mediate repression by competing with Sp1 for BTE binding. Thus, the characterization of this protein expands the repertoire of BTEB-like members of the Sp1 family involved in transcriptional repression. Furthermore, our results suggest a mechanism of repression for BTEB3 involving direct repression by the N terminus via interaction with mSin3A and HDAC-1 and competition with Sp1 via the DNA-binding domain.

Sp1-like proteins, defined by the presence of three highly homologous C-terminal zinc finger motifs and variant N-terminal domains, are emerging as important regulators of cell homeostasis. Promoters containing Sp1-like sites are essential for the expression of numerous genes necessary for cell cycle progression (1–3), DNA synthesis (4), and other cell processes (5–8), and studies have shown that certain Sp1-like proteins induce apoptosis (9), cell growth inhibition (10–12), differentiation (13, 14), and carcinogenesis (15). In addition, the disruption of some Sp1-like genes in mice shows that these proteins are critical for normal development (12, 16–18). Thus, understanding how Sp1-like proteins bind DNA and regulate transcription is important to uncover the molecular mechanisms underlying a large number of cellular events.

The existence of at least 17 different Sp1-like proteins offers a significant challenge for understanding how individual members regulate gene expression in a tissue-, cell-, and promoterspecific manner. One mechanism leading to specificity among Sp1-like proteins is a differential pattern of expression. For instance, Sp1, TIEG2, and BTEB1 are ubiquitously expressed, whereas the KLF proteins are restricted to certain tissues. Specificity among Sp1-like proteins is also dictated by recognition of DNA. For example, the Sp proteins preferentially bind GC sites (19, 20) whereas the KLF subgroup prefers the CA site (21–23). Interestingly, co-expressed Sp1-like proteins exhibiting similar binding specificity, such as Sp1 and Sp3, but often display opposite transcriptional regulatory properties (24, 25). Thus, activators and repressors that bind to the same sequence may have evolved to turn on and off a discrete set of promoters by competing for this site. In this regard, emerging evidence indicates that Sp1-like proteins are able to compete for DNA binding, such as has been reported for BTEB1/Sp1/GKLF (8, 26) and BKLNF/EKLF (27). Together, the results of these mechanistic studies on competition between Sp1-like proteins validate many of the predictions derived from sequence homology data, suggesting that these proteins regulate similar sequences in vivo.

The molecular mechanisms behind the function of the Sp1-like activators have been extensively reported in the literature. For example, both Sp1 and EKLF have been shown to activate transcription by interacting with co-activators (13, 28–32). In contrast, the molecular mechanisms involved in the function of Sp1-like repressor proteins are less defined. The Sp1-like proteins involved in transcriptional repression include the TIEG proteins (9, 10, 33), Sp3 (24, 25), BKLNF and BKLNF2/KLF8 (21, 34), GKLNF (8), and Ap2-rep (35). However, only BKLNF and a closely related protein, BKLNF2/KLF8, have been shown to as-

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All these proteins bind similar sequences, an important question that remains is whether the mechanism of repression is similar between all Sp1-like repressors or whether Sp1-like subfamilies are defined by distinct mechanisms of repression.

In this study, we have pursued this question by characterizing the human transcriptional repressor BTEB3, a novel Sp1-like protein that is significantly related to BTEB1. A partial sequence of this protein was recently published with the name of NSLP1 from our laboratory (37), but in light of its sequence similarity and functional conservation with BTEB1, we have renamed it BTEB3. This study focuses on the characterization of BTEB3 as a transcriptional repressor with two mechanisms of action: competition with the activator Sp1 and interaction with the co-repressor mSin3A and the histone deacetylase protein HDAC-1. These results expand our understanding of the functional properties of BTEB-related Sp1-like proteins and suggest that these proteins have evolved at least in part to balance the activating function of Sp1. Our data also provide evidence that different subfamilies of Sp1-like transcriptional repressor proteins, such as BTEBs and BKLFs, may function via association with distinct co-repressors.

EXPERIMENTAL PROCEDURES

Cell Culture and Plasmid Construction—The Chinese hamster ovary (CHO) epithelial cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD) and cultured in Ham’s F-12 medium, supplemented with 5% fetal bovine serum, 5% newborn calf serum, 100 units/ml streptomycin, and 100 units/ml penicillin (Life Technologies, Inc.). Standard molecular biology techniques were used to clone BTEB3 and its various deletion mutants into the pcDNA3.1/His (Invitrogen, Carlsbad, CA), pcMVtag2 (Strategene, La Jolla, CA), pEGEX (Amerham Pharmacia Biotech), and pM GAL4-DBD (CLONTECH, Palo Alto, CA) vectors for expression as His-tagged, FLAG-tagged, GST fusion, or GAL4 proteins, respectively. All constructs expressing the zinc finger domain included the BTEB3 bipartite nuclear localization signal sequence found immediately upstream (data not shown). For reporter assays, we cloned five tandem GAL4 DNA binding sites upstream of the thymidine kinase promoter or six tandem BTE sites (pBTE6) upstream of the adenovirus major late promoter in the pGL3 basic firefly luciferase reporter vector. Renilla luciferase reporter vectors were verified by sequencing.

To determine expression levels of GAL4 constructs, immunocomplexes were collected by centrifugation, washed five times with lysis buffer, and subjected to Western blot analysis as described above using anti-BTEB3, anti-mSin3A, or anti-HDAC1 polyclonal antibodies (Santa Cruz Biotechnology). To determine the expression level of GAL4 reporter constructs, immunocomplexes were separated on SDS-PAGE and autoradiography was performed as for GST pull-down assays.

GST Pull-down Assays—To determine the GST fusion proteins used in these studies, BL21 bacteria were induced with isopropyl-1-thio-β-D-galactopyranoside to express fusion proteins carrying various deletions of BTEB3 and the recombinant fusion proteins were purified using GST-Sepharose beads according to manufacturer’s suggestions (Amersham Pharmacia Biotech). The [35S]methionine-labeled full-length mSin3A and HDAC-1 proteins were produced by in vitro transcription using the TNT-coupled transcription/translation system under the conditions described by the manufacturer (Promega). The in vitro translated proteins were incubated with 2 μg of GST or GST fusion proteins and glutathione-Sepharose beads in lysis buffer for 4 h at 4 °C. Complexes were collected by centrifugation, washed with lysis buffer and separated by SDS-PAGE. The gel was treated with AutoFluor (National Diagnostics, Atlanta, GA), dried, and exposed for autoradiography at ~80 °C.

RESULTS

BTEB3 Is a BTE-binding, Sp1-like Transcriptional Repressor—Pull-length mouse and human cDNAs encoding BTEB3, a new member of the BTEB subfamily of Sp1 proteins, were cloned based on sequence comparisons of a partial cDNA with the IMAGE Washington University expressed sequence tag data base. Sequence analysis revealed that the sequences of our human and mouse BTEB3 proteins are identical to recently published sequences for human RPLAT-1 and FKLF-2 (39, 40) and mouse BTEB3 and KLF13 (39, 41), respectively. Results from Northern blot analysis showed that human BTEB3 is ubiquitously expressed (data not shown) and are in agreement with previously published reports on this gene (41, 42).


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Fig. 1. BTEB3 binds the GC-rich BTE DNA element. A gel shift assay was performed using the three zinc finger motifs of BTEB3 expressed as a GST fusion protein (BTEB3 ZF) and a double-stranded oligonucleotide probe containing the wild type BTE consensus site. Lane 1, BTE probe alone; lane 11, 170 ng of GST protein; lanes 2–7, 170 ng of BTEB3 ZF GST fusion protein with wild type BTE probe (lanes 2 and 5), 500-fold molar excess cold wild type BTE probe (lane 3), 500-fold molar excess AP-1 probe (lane 4), anti-GST antibody (lane 6), and anti-BTEB3 antibody (lane 7); lanes 8–10, 170 ng of Sp1 ZF GST fusion protein with wild type BTE probe (lane 8), anti-GST antibody (lane 9), and anti-BTEB3 antibody (lane 10). The specific complexes are indicated on the right. Note that the anti-GST antibody supershifts both the BTEB3 and Sp1 fusion protein complexes, whereas the anti-BTEB3 antibody supershifts the BTEB3 complex only.

In addition, we identified a functional nuclear localization signal sequence located immediately upstream of the zinc finger domain (data not shown).

Outside of the zinc finger motif, BTEB3 does not share significant sequence homology with other Sp1-like proteins except BTEB1. The similarity between the zinc finger domains of BTEB1 and BTEB3 predict that BTEB3 will bind to GC-rich sequences, such as the BTE. The BTE site is a regulatory element of the gene for CYP1A1 (43, 44) and has been described as the preferred binding site of BTEB1 (45). The Sp1-like proteins BTEB1, Sp1, and GKLF have been shown to regulate transcription of BTE-containing promoters (8, 26). To test whether BTEB3 binds the BTE site, we performed gel shift assays using the zinc finger motifs of BTEB3 and a BTE probe as described under “Experimental Procedures.” Fig. 1 shows that both BTEB3 (lanes 2 and 5) and Sp1 (lane 8) zinc finger motifs, but not GST alone (lane 11), bound the BTE site. BTEB3 DNA binding activity was competed using an excess of unlabeled BTE probe (lane 3) but not unlabeled AP-1 probe (lane 4). A supershift of the BTEB3-BTE complex using anti-GST and anti-BTEB3 antibodies (lanes 6 and 7, respectively) demonstrated that the observed shift was due to the fusion protein and not trace amounts of bacterial proteins that may have been co-puriﬁed. A similar supershift of the Sp1-BTE complex was observed only when using the anti-GST antibody, but not the anti-BTEB3 antibody (lanes 9 and 10, respectively). These studies show that BTEB3 speciﬁcally bound to the consensus BTE DNA element in vitro.

To characterize the transcriptional regulatory activity of BTEB3, we used the GAL4-based reporter system as described under “Experimental Procedures.” Fig. 2A shows that the N terminus of BTEB3 strongly repressed transcription in a dose-dependent manner when tethered to DNA through the heterologous DNA-binding domain of GAL4.

BTEB3 Repressed Transcription in a TSA-sensitive Manner—Subsequently, we investigated the molecular mechanisms underlying the transcriptional repression activity of BTEB3. A major discovery toward understanding the molecular mechanisms of transcriptional repressors has been the finding that transcriptional regulatory domains interact with histone deacetylase containing complexes. To determine if the N terminus of BTEB3 utilizes histone deacetylase activity to repress transcription, we performed the GAL4 reporter assay in the presence of TSA, a well known histone deacetylase inhibitor. Fig. 2B shows that TSA, at 100 ng/ml, relieved the repression mediated by the BTEB3 N terminus by 2-fold, in a manner similar to the well characterized transcription factor Mad1 (46). The results of this experiment suggest that histone deacetylase activity is important for BTEB3-mediated repression. Therefore, we hypothesize that the N terminus of BTEB3 interacts with co-repressors that are important for the recruitment of histone deacetylases.

BTEB3 Interacted with the Co-repressors mSin3A and HDAC-1—To further explore the mechanism(s) behind BTEB3 repression, we determined whether BTEB3 associated with histone deacetylase containing co-repressor complexes. For this purpose, we made a GST fusion protein expressing the N terminus of BTEB3 and performed GST pull-down assays using metabolically labeled CHO cell extracts. Preliminary results revealed that the N-terminal domain of BTEB3 specifically pulled down several peptides. Two consistently present peptides were 160- and 55-kDa proteins, the molecular sizes expected for the co-repressors mSin3A and HDAC-1, respectively, which are well characterized members of a multi-subunit co-repressor complex (data not shown). We analyzed this putative interaction by performing immunoprecipitation assays combined with Western blot analysis. Fig. 2C demonstrates that endogenous BTEB3, mSin3A, and HDAC-1 were co-precipitated by an anti-BTEB3 antibody (lane 1) and that the molecular weights of the proteins recognized by mSin3A- and HDAC-1-specific antibodies correlated with the proteins detected by the GST pull-down assay. To further probe the relevance of this interaction, we transfected plasmids expressing FLAG-tagged BTEB3 full-length (BTEB3 FL) or zinc finger domains (BTEB3 ZF) into CHO cells and performed immunoprecipitations using an anti-FLAG antibody. Fig. 2C shows that mSin3A and HDAC-1 were specifically precipitated from cells transfected with BTEB3 FL (lane 3), but not BTEB3 ZF (lane 4) or control cells transfected with the parental FLAG vector (lane 2). Taken together, these data show that the N terminus of BTEB3 mediated interaction with co-repressors involved in chromatin remodeling, namely mSin3A and HDAC-1.

BTEB3 Repressed Transcription via Three Separable Repression Domains—To determine if the N terminus of BTEB3 contained specific repression domains, GAL4 fusion constructs containing various deletions of N- or C-terminal regions of BTEB3 were generated (Fig. 3A). Fig. 3B shows that the maximal repression activity of the N terminus of BTEB3 was ~35-fold (9.72 ± 0.36 versus 0.26 ± 0.0091, GAL4 DBD versus N terminus). In addition, constructs containing aa 1–24, 55–74, and 75–114 display 11-, 2.6-, and 3.3- fold repression activity,
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respectively (0.85 ± 0.045, 3.75 ± 0.31, and 2.93 ± 0.29, respectively). For the remainder of the paper, we will refer to these regions of BTEB3 as follows: aa 1–24 as repression domain 1 (R1), aa 55–74 as repression domain 2 (R2), and aa 75–114 as repression domain 3 (R3). Even though R1, R2, and R3 are separable repression domains, it appears that a combination of R1 with R2, and/or R3 is necessary for full repression in this assay. The repression activity achieved by R1 in combination with R2 (aa 1–84; 0.27 ± 0.05) or R1 in combination with both R2 and R3 (aa 1–114; 0.23 ± 0.05) was 35- and 41-fold, respectively, similar to the repression activity of the entire N terminus. The combination of R2 and R3 resulted in only 7-fold repression (aa 55–113; 1.35 ± 0.12). Constructs containing the C terminus, aa 25–54, and aa 115–168, on the other hand, displayed no significant transcriptional regulatory activity (7.02 ± 0.77, 6.88 ± 0.74, and 6.51 ± 0.36, respectively). To control for DNA binding and expression of the GAL4 fusion constructs, we performed gel shift (Fig. 3C) and GAL4 immunoprecipitation (Fig. 3D) assays and normalized the repression activity observed to the level of expression. This study demonstrates that the N terminus of BTEB3 contained at least three separable repression domains. At no point did we find activation of transcription in this assay, in contrast to the previously published activation by BTEB3 (40–42).

The ability of these repression domains to function in vivo together with the native BTEB3 DNA binding domain was tested with a reporter plasmid containing BTE binding sites, as described under “Experimental Procedures” (Fig. 4A). Fig. 4B shows that full-length BTEB3 as well as each repression domain inhibited the BTE promoter ~4-fold (normalized to expression level). To control for expression and DNA binding, we performed gel shift and Western blot assays (Fig. 4C). Thus, these studies demonstrate that BTEB3 repression domains not only repressed transcription in GAL4-based assays, but also repressed a BTE-containing promoter in vivo through the native BTEB3 DNA binding domain.

We next tested whether R1, R2, or R3 associate with co-repressor proteins. For this purpose, we made GST fusion proteins containing each domain and performed GST pulldown assays using in vitro translated mSin3A and HDAC-1. Fig. 5D demonstrates that each of the repression domains of BTEB3 directly interacted with mSin3A, but not HDAC-1. These results are consistent with a model in which mSin3A acts as a bridge between the transcription factor and HDAC-1, as has been shown for Mad1 (46). This experiment, combined with the results of the immunoprecipitation (Fig. 2C) and TSA assays (Fig. 2B), provides evidence that BTEB3 is able to repress transcription directly via the recruitment of histone deacetylase associated co-repressors. Therefore, we conclude that BTEB3, differently from the BKL family subfamily, defines a novel class of Sp1-like proteins that repress transcription by interacting with mSin3A and HDAC-1.

Competition between BTEB3 and Sp1 for BTE—Competition for DNA binding has been shown to be an important mechanism of transcriptional regulation (8, 26, 27). To test whether BTEB3 and Sp1 have different affinities for the BTE site, we compared the BTE binding affinities of Sp1 and BTEB3 zinc fingers. Equal molar quantities of recombinant zinc finger Sp1
or BTEB3 proteins were incubated with increasing amounts of end-labeled BTE probe in a gel shift assay (Fig. 5A). We measured the dissociation constant ($K_d$) of Sp1 as 1.07 nM ± 0.06 versus 0.81 nM ± 0.12 for BTEB3. The Sp1 $K_d$ was consistent with previously reported measurements of 0.5–3 nM (8, 26). The results show that BTEB3 has an affinity for the BTE site similar to that of Sp1 in vitro.

Due to the similarity of the affinities that Sp1 and BTEB3 exhibited for BTE binding in vitro, we hypothesized that BTEB3 was able to compete with Sp1 for DNA binding and
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The dissociation constant is 1.07 nM for BTEB3.

Therefore, antagonize the activator function of Sp1. To test this hypothesis, we transfected CHO cells, in which the major BTE binding protein is Sp1 (8), with increasing amounts of BTEB3. Whole cell extracts were obtained and used with the BTE probe in a gel shift assay. Untransfected CHO cells (control) exhibited a strong Sp1 BTE binding activity (Fig. 6A, lanes 1 and 7), as demonstrated by a supershift using a Sp1-specific antibody (lane 13). Fig. 6A shows that, as the expression and BTE binding activity of BTEB3 increased, the BTE binding activity of Sp1 decreased, suggesting that BTEB3 was indeed competing with Sp1 for binding to the BTE site (lanes 2–6). In addition, expression of the zinc finger domains of BTEB3 alone appeared to decrease Sp1 binding to the BTE site (lanes 8–12) to the same extent as the full-length protein. To control for expression of Sp1 and BTEB3, Western blot analysis was performed using anti-Sp1 and anti-BTEB3 antibodies. Fig. 6B shows that, as BTEB3 (ZF or FL) expression increased, Sp1 protein levels remained the same and that BTEB3 FL and ZF proteins were expressed at comparable levels. Thus, both the full-length protein and the zinc finger domain alone were capable of competing with Sp1 for binding of the BTE promoter.

Subsequently, we determined the effect of this competition on the activity of a BTE-containing promoter. Fig. 6C shows that full-length BTEB3 repressed transcription at a BTE-containing promoter in a dose-dependent manner. The maximum repression observed was 4-fold (1.87 ± 0.46 versus 7.30 ± 0.87, BTEB3 FL versus control) and is in agreement with the results presented in Fig. 4B. The repressor activity of the zinc finger domain alone was then directly compared with the repression activity of full-length BTEB3 when both peptides were expressed in CHO cells at similar levels. Because this construct contains only the functional nuclear localization signal sequence and DNA-binding domain, but lacks the three transcriptional regulatory domains of BTEB3 and does not interact with co-repressors (see Fig. 2C, lane 4), any repression activity observed should be due to competition with endogenous BTE-binding activator proteins. Fig. 6C shows that, similarly to full-length BTEB3, the zinc finger domain alone repressed transcription of the BTE promoter in a dose-dependent manner. However, the maximal repression achieved by this domain was only 2-fold (4.63 ± 0.48 versus 8.12 ± 0.43, BTEB3 ZF versus control) compared with 4-fold repression observed with full-length BTEB3. Taken together, the data demonstrate that BTEB3 has the ability to repress transcription by displacing the activator Sp1 through competition for DNA binding. Therefore, we conclude that BTEB3 not only can repress transcription directly via N-terminal repression domains and co-repressors but also by competing with the activator Sp1.

**DISCUSSION**

Here, we have characterized the transcriptional repressor and DNA binding activities of BTEB3, a novel member of the BTEB subfamily of Sp1-like proteins. We demonstrated that the BTEB3 N-terminal repressor activity was TSA-sensitive and associated with mSin3A and HDAC-1 *in vivo* (Fig. 2). However, TSA did not completely reduce BTEB3-mediated repression, a phenomenon also observed for the well-characterized mSin3A-interacting protein Mad1 (46). Interestingly, mSin3A lacking the HDAC-1 interacting domain can still repress (46, 47), which indicates that other mechanisms participate in mSin3a-mediated repression. Thus, in a similar manner, other histone deacetylase-independent co-repressors or chromatin-remodeling complexes may contribute to BTEB3 repression activity.

The N terminus of BTEB3 contains three separable repression domains (R1, R2, and R3) (Fig. 3). R1 is separated from R2 by a linker and exhibits homology to the R1 domains of TIEG proteins, which bind to mSin3A (50), suggesting that it may function as a distinct repressor domain in *vivo*. In contrast, since a linker does not separate R2 and R3, these may comprise a single repression domain. Fig. 4 shows that each domain silences transcription of a BTE-containing promoter via the native DNA binding domain to the same extent as the full-length protein. This is different from the data of the GAL4 assay (Fig. 3), which show that each domain is less potent than the entire N terminus. This discrepancy may be due to differences in the promoter context. In addition, different DNA binding domains and associated proteins may influence the function of these domains. Currently, it is difficult to define which of these possibilities is operating *in vivo*. Fig. 4D demonstrates that the repression domains of BTEB3 bind mSin3A, but not HDAC-1, supporting a model in which BTEB3 directly interacts with mSin3A, to recruit HDAC-1. These data are consistent with results show-
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A whole cell extract of CHO cells, transfected with increasing amounts of His-tagged FL BTEB3 or the ZF of BTEB3 constructs, were used in a BTE-binding gel shift assay. The specific protein-DNA complexes are indicated on the right. Untransfected CHO cells exhibit Sp1 BTE binding activities (lanes 1, 7, and 13). A supershifted band is observed in lane 13 when anti-Sp1 antibody is used. As the BTE binding activity of FL BTEB3 (lanes 2–6) or ZF BTEB3 (lanes 8–12) increases, the binding of Sp1 decreases. B, Western blot analysis of CHO cells transfected with increasing amounts of FL or ZF BTEB3 shows steady expression of Sp1 while the expression of FL or ZF BTEB3 increases. Note that the full-length protein and the zinc fingers alone are expressed at comparable levels. C, BTEB3 FL or ZF were co-transfected into CHO cells along with pBTEg luciferase activity was measured as a ratio of pBTEg/pBTE0 and was normalized to expression level. Note that overexpression of the full-length BTEB3 results in approximately 4-fold repression, whereas the zinc finger domain alone exhibits 2-fold repression.

FIG. 6. BTEB3 competes with and antagonizes Sp1 activation of a BTE-containing promoter. A, whole cell extracts of CHO cells, transfected with increasing amounts of His-tagged FL BTEB3 or the ZF of BTEB3 constructs, were used in a BTE-binding gel shift assay. The specific protein-DNA complexes are indicated on the right. Untransfected CHO cells exhibit Sp1 BTE binding activities (lanes 1, 7, and 13). A supershifted band is observed in lane 13 when anti-Sp1 antibody is used. As the BTE binding activity of FL BTEB3 (lanes 2–6) or ZF BTEB3 (lanes 8–12) increases, the binding of Sp1 decreases. B, Western blot analysis of CHO cells transfected with increasing amounts of FL or ZF BTEB3 shows steady expression of Sp1 while the expression of FL or ZF BTEB3 increases. Note that the full-length protein and the zinc fingers alone are expressed at comparable levels. C, BTEB3 FL or ZF were co-transfected into CHO cells along with the pBTEg luciferase activity was measured as a ratio of pBTEg/pBTE0 and was normalized to expression level. Note that overexpression of the full-length BTEB3 results in approximately 4-fold repression, whereas the zinc finger domain alone exhibits 2-fold repression.

...ing that mSin3A and HDAC-1 co-precipitate with BTEB3 (Fig. 2C). Thus, BTEB3 represses transcription by a mechanism involving the recruitment of the HDAC-1-mSin3A complex via three N-terminal repression domains.

Since Sp1 is ubiquitously expressed, displacing it from promoters by competition may be a significant mechanism of repression. Fig. 5 shows that BTEB3 binds the BTE element with an affinity comparable to Sp1, suggesting that competition between the two proteins is possible. Fig. 6 shows that BTEB3 does compete with Sp1 to antagonize its activation function. These data support a model in which competition with Sp1 via the zinc finger domain can contribute to the repressor function of BTEB3. To evaluate the contribution of each mechanism to the overall repression of BTEB3, we created a BTEB3 mutant containing the zinc fingers and evaluated its ability to compete with and antagonize Sp1. Fig. 6A shows that this domain can compete with Sp1 similar to the full-length protein. It also retains its ability to repress a BTE-containing promoter, although to a lesser degree than the full-length protein (Fig. 6C). Since the BTEB3 zinc finger domain alone is not able to interact with co-repressor proteins (Fig. 2C) but retains partial repression activity, this further supports the idea that BTEB3 represses transcription by competing with Sp1. Thus, it is likely that, in vivo, the competition for DNA binding as well as direct repressor function are acting together to suppress transcription at promoters activated by Sp1. However, the contribution of these mechanisms to the overall repression activity may vary according to promoter context, co-repressor availability, or signaling. This model is attractive since a potent repression mechanism of this type may be required to counterbalance Sp1-mediated activation, the major GC binding activity detected in most mammalian cells. Indeed, several Sp1-like proteins may function according to this model, including BTEB1 (26), Sp3 (25), GKLF (8), and TIEG2. 2 In addition, like BTEB3, some of these proteins also contain an N-terminal repressor activity. Thus, it is likely that both of these mechanisms of regulation may be a common motif among a subset of Sp1-like proteins.

Recently, BTEB3 has been reported to display activator function (41, 42). In this regard, although we do not rule that this protein may have activator function, the detailed characterization reported here strongly supports a repressor role for this gene. Interestingly, other Sp1-like proteins including BTEB1 (45, 46) and TIEG2 (10, 33, 49) display both activator and repressor function depending on the promoter context. Indeed, the activation previously reported for BTEB3 was observed in a different promoter context and includes the SV40 (41), β-globin (40), and RANTES (42) promoters. In addition, the cell lines used for these studies may also express other factors that have an effect on both the DNA binding and transcriptional regulatory activity of BTEB3. These differences in the systems need to be taken into consideration when evaluating the data and will require further investigation to better understand BTEB3-mediated transcriptional control.

In summary, the fact that BTEB3 can repress transcription through at least two mechanisms makes it a good candidate for a repressor that can counterbalance the potent activator function of Sp1 on GC promoters. More importantly, we show that BTEB3 represses transcription by a mechanism different from that of BKLF proteins, suggesting that Sp1-like proteins can be classified into subgroups based on the mode of repression. However, it remains possible that these proteins interact with different co-repressors under different conditions and future experiments may better clarify these data. Thus, our results support the hypothesis that Sp1-like proteins with similar DNA binding domains but different transcription regulatory properties have evolved to provide fine-tune gene expression regulation on certain GC-rich sequences present in mammalian promoters.

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