The Leukemia-associated Protein Btg1 and the p53-regulated Protein Btg2 Interact with the Homeoprotein Hoxb9 and Enhance Its Transcriptional Activation*

(Received for publication, August 23, 1999, and in revised form, October 11, 1999)

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BTG1 and BTG2 belong to a family of functionally related genes involved in the control of the cell cycle. As part of an ongoing attempt to understand their biological functions, we used a yeast two-hybrid screening to look for possible functional partners of Btg1 and Btg2. Here we report the physical and functional association between these proteins and the homeodomain protein Hoxb9. We further show that Btg1 and Btg2 enhance Hoxb9-mediated transcription in transfected cells, and we report the formation of a Hoxb9-Btg2 complex on a Hoxb9-responsive target, and the fact that this interaction facilitates the binding of Hoxb9 to DNA. The transcriptional activity of the Hoxb9-Btg complex is essentially dependent on the activation domain of Hoxb9, located in the N-terminal portion of the protein. Our data indicate that Btg1 and Btg2 act as transcriptional cofactors of the Hoxb9 protein, and suggest that this interaction may mediate their antiproliferative function.

The BTG gene family, whose founding member is BTG1 (B-cell translocation gene 1) (1, 2), is composed, in vertebrates, of at least seven distinct members: BTG1, BTG2/TIS21/PC3, BTG3/TOB5/ANA, TOB, TOB4, B9.10, and B9.15. The defining feature of this family is the presence of two short conserved domains (box A and box B) separated by a spacer sequence of 20–25 non-conserved amino acids (3). These proteins are found in organisms from nematodes to humans, and a large body of evidence suggests that Btg proteins may be mediators of multiple antiproliferative activities. Btg proteins are involved in cell growth control and differentiation in models such as TGF-β-treated, O-tetradecanoylphorbol-13-acetate-treated, and neuron-cell birthday in the mouse embryo (4–7). Furthermore, BTG2 expression is regulated by p53, and its inactivation in embryonic stem cells leads to the disruption of DNA damage-induced G2/M cell-cycle arrest (5). It had also been suggested that BTG2 could be involved in programmed PC12 cell death (8, 9). The functional specificity and selectivity of the different members of the Btg family could be achieved by the interaction with different cellular targets; Tob can associate with the ErbB2 growth factor receptor, which may modulate the signal elicited by epidermal growth factor (10); it has been shown that both Btg1 and Btg2 interact with a protein-arginine N-methyltransferase (Prmt1), and modulate its activity positively (11); and we have demonstrated the physical interaction of both Btg1 and Btg2 with the mouse protein Caf1 (CCR4-associated factor 1) (12), the mouse homolog of the yeast yCAF/POP2 gene, which regulates the expression of a number of genes involved in cell-cycle regulation and progression. In this study, we report the characterization of another Btg-associated protein, the Hoxb9 homeotic protein, which is a member of a large family of transcription factors that share a similar DNA-binding motif known as the homeodomain, an evolutionarily conserved sequence of 60 amino acids. The Hox family of homeodomain-containing transcription factors regulates cell fates during the development of metazoans (13). Despite their functional specificity in vivo, a large number of Hox proteins (up to 38 in mammals) appears to bind in vitro to a restricted set of very similar DNA sites with a similar degree of affinity (14). There is considerable evidence that the diversity of the functions of Hox in vivo depends not only on their DNA-binding properties, but also on transcriptional co-factors (15–17). In the present study, we show that Btg1 and Btg2 interact with Hoxb9, and that the homeodomain is involved in these interactions. We also show that Btg1 and Btg2 can cooperate with Hoxb9 to activate transcription in transfected cells. The transcriptional activation domain of Hoxb9 was identified by deletion analysis, and was mapped to the N-terminal region of the protein. Our results indicate that Btg1 and Btg2 may function as cofactors for Hoxb9-mediated transcription.

EXPERIMENTAL PROCEDURES

Yeast Expression Constructs—The Btg1 and Btg2 “bait” for the yeast two-hybrid system was based on the pPC62 yeast expression vector (18). pPC62 was cut with SalI and XbaI, and ligated with SalIXbXbI BTG1 and BTG2 human cDNA, obtained by PCR, giving pPC62BTG1 and BTG2 encoding fusion proteins, consisting of the Gal4 DNA-binding domain fused to Btg1 and Btg2. The BTG1 deletion mutants, constructed as Gal4 fusions, which have already been described (12), are outlined in Fig. 1. The pPC86HOXB9/185–250 was generated cloning the fragment containing the Hoxb9 homeodomain (amino acid residues 180–246) obtained by PCR from the full-length coding sequence of mouse HOXB9 in frame with the Gal4 transactivation domain of the pPC86 plasmid. The Hox2.5 plasmid, containing the full-length coding sequence of mouse HOXB9 gene, was kindly provided by F. Rudde (Yale University, New Haven, CT).

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The supernatants were assayed for Cat protein production and transfection. The transfected DNA (1 μg) was introduced into the yeast strain Saccharomyces cerevisiae (YEp13 plasmid) containing the GAL1 gene (yeast reporter gene) under the control of the galactose-inducible GAL1 promoter. The transformed yeast cells were grown in a shake flask at 30 °C on an Leu/Trp/His amino acid-depleted DOB medium, the transformants were tested for β-galactosidase activity using a yeast colony-filter assay. Positive (blue) colonies were grown in selective medium for 2 days to recover the prey plasmid. An Electromax DH10B bacterial strain was used to recover the expression plasmid from the selected transformed yeast (18).

**Bacterial Expression Constructs and Production of Recombinant Proteins**—To generate the bacterial expression vector for Hoxb9, its full-length coding sequence was inserted into the pGEX-expression vector (Amer sham Pharmacia Biotech) in-frame with the glutathione S-transferase (GST) coding sequence. GST and GSTHoxb9 proteins were expressed in Escherichia coli DH5α, purified on glutathione-Sepharose beads (Amer sham Pharmacia Biotech) and quantified using the Bradford method. Recombinant 6His vector for human Btg2 was constructed by inserting the cDNA into the BamHI site of pQE30 vector (Qiagen). This placed the Btg2 coding sequence in frame with six sequential histidine residues. Recombinant polyhistidine-tagged Btg2, expressed in M15 (pREP4), was purified from soluble lysates with a nickel chelation affinity column (Qiagen) according to the manufacturer’s recom- mended protocol. SDS-PAGE and Coomassie staining were used to confirm the integrity of the full-length fusion proteins. The 6HisFlag protein was provided by R. Rimokh (U453 INSERM, Lyon, France).

**Mammalian Reporter Plasmids**—The pHBS-TK-CAT plasmid was generated by inserting two copies of a SalI-SalI double-stranded oligonucleotide containing the Hoxb9-responsive element HBSII of the N-CAM gene promoter, into the SalI site of the plasmid pHLECAT2. HBSII sense strand consisted of 5′-gtgCAGAAATTCCTGTTATAG-GAACGTGCAC-3′. The core binding site is shown in bold letters. The pG4-TK-CAT reporter plasmid contains six GAL4 consensus elements upstream from the thymidine kinase (TK) promoter region, fused to the CAT gene.

**Mammalian Expression Constructs**—All the mammalian expression constructs used were derivatives of the pcDNA3 promoter-driven expression vector pSG5 (Stratagene). The pSG5Flag plasmid, which was derived from pSG5 (12), contains the Flag peptide sequence (IBI Flag system; Eastman Kodak Co.). The full-length Hoxb9 cDNA and the fragment coding for the region containing amino acids 79–250 and 185–250, obtained by PCR, were subcloned into the Xhol/Spe1 sites of the pSG5Flag vector in order to generate pSG5FlagHoxb9, pSG5FlagHoxb9/79–250, and pSG5FlagHoxb9/185–246. pSG5FlagPOLL was kindly provided by R. Rimokh (U453 INSERM, Lyon, France). The expression constructs producing Hoxc8 and Hoxd9 under SV40 promoter control (pTL1C8 and pSGH4C) were kindly provided by M. Councey (CERN, Switzerland) and M. Rimokh (U453 INSERM, Lyon, France). The expression constructs producing Hoxc8 and Hoxd9 under SV40 promoter control (pTL1C8 and pSGH4C) were kindly provided by M. Councey (CERN, Switzerland) and M. Rimokh (U453 INSERM, Lyon, France). The expression constructs producing Hoxc8 and Hoxd9 under SV40 promoter control (pTL1C8 and pSGH4C) were kindly provided by M. Councey (CERN, Switzerland) and M. Rimokh (U453 INSERM, Lyon, France).

**RESULTS**

**Both Btg1 and Btg2 Interact with Hoxb9**—To study the role of Btg proteins in the control of cell growth, we focused on Btg1 and Btg2, which are closely related proteins sharing over 60% of amino acid identity, and tried to identify interacting proteins using the yeast two-hybrid screen (21). A fusion protein made of the DNA-binding domain of Gal4 and the full-length Btg1 protein was used as a bait to screen a 14.5-day-old mouse embryo cDNA library cloned in pPC86, as already described (12). Two out of clones producing β-galactosidase were further analyzed, and one of them was found to be identical to the CAF1 mouse gene (12). The second one, which we named C2, was found to encode a protein with a sequence identical to that of the homoeotic protein Hox9. Clone C2 begins at nucleotide 310 and at amino acid 78 according to Malicki (EMBL/GenBank™ accession number S66865) (22). The cDNA encompassing the entire mouse Hox9 ORF was cloned from F2.5 plasmid kindly provided by F. Ruddle.

To outline the region of Btg1 that mediates its interaction with Hoxb9, we made a series of Btg1 truncation constructs (Fig. 1). Using the yeast two-hybrid system, we found that the Btg1 region between residues 1 and 38 was sufficient for interaction with Hoxb9 (1). When tested in two-hybrid system, the 4.2 clone product also interacted with the pPC82BTG2 expressed protein. Similarly, using a truncated fusion protein containing the homeodomain alone, we found that Hoxb9 ho-
meodomain was sufficient for binding with Btg1 and Btg2. The results of these assays are summarized in Fig. 1.

**Hoxb9 Interacts with Btg1 and Btg2 in Vitro**—To confirm the interaction of Btg1 and Btg2 with Hoxb9 in vitro, we performed a Far-Western blot analysis. Purified GST, GSTHoxb9, and an induced bacterial extract containing GSTHoxb9 were subjected to SDS-PAGE, transferred from the gel to nitrocellulose, and probed with $^{35}$S-labeled Btg1 and Btg2 proteins. As shown in Fig. 2, specific hybridization was observed with GSTHoxb9, but not with control GST. In addition, incubation with $^{35}$S-labeled GSTHoxb9 proteins, we did analogous experiments with Hox9, a paralog of Hoxb9 and Hoxc8, which is a member of a different paralog group. Under the conditions of our experiments, only Hoxc8 bound with Btg2 protein in vitro (Fig. 3).

**Btg2 Facilitates Hoxb9 Binding to Its Target DNA, and Forms a Ternary Complex with Hoxb9 and DNA**—Several in vitro and in vivo studies support the hypothesis that homeobox gene products modulate the expression of adhesion molecule genes (23–27). The promoter for the neural cell adhesion molecule (N-CAM) contains a 47-bp region, including two potential homeodomain binding sites (HBSI and HBSII) (25). These studies showed that HBSII is a target site for the binding and transcriptional control of the N-CAM promoter by the homeoprotein Hoxb9. To determine whether Hoxb9 and Btg proteins interact directly on DNA, we tested the influence of Btg on the in vitro binding of Hoxb9 to the HBSII sequence, using the full-length Hoxb9 protein as a GST-fusion in gel-mobility shift experiments, with a single HBSII site as a probe (Fig. 4A). A retarded complex corresponding to GSTHoxb9-DNA was observed (Fig. 4B, lane 4). This was specifically competed by unlabeled HBSII (lanes 6 and 7), but not by the mutated HBSII oligonucleotide (lanes 8 and 9). A retarded complex was found when the GST protein was used (lane 1). The GSTHoxb9-DNA complex was supershifted by the anti-GST antibody (lane 5). When 6HisBtg2 was included in the Hoxb9-DNA binding incubation mixture, an additional slow mobility complex was seen. This indicates that a DNA-Hoxb9-Btg2 complex was formed (lane 10). The complex exhibited the same DNA-binding specificity, as shown by the occurrence of specific competition with an excess of unlabeled oligonucleotide (lane 11). In contrast, no slow migrating retarded band was observed with the 6His-control protein, 6HisFlrg (lane 12). With 6HisBtg2 or 6HisFlrg alone, no shifted complexes were observed in our EMSA conditions (see lanes 2 and 3). Importantly, the addition of Btg2 enhanced Hoxb9 binding activity, as revealed by a stronger Hoxb9-DNA complex (Fig. 4B, compare lanes 4 and 10), indicating that the Hoxb9-Btg interaction increased the affinity of Hoxb9 for HBSII sites.

**Btg1 and Btg2 Enhance Hoxb9-dependent Transcription**—In order to study both the transcriptional properties of Hoxb9 and the possible functional interactions of Btg1 and Btg2 with Hoxb9, we performed transient-transfection assays in HeLa cells. We constructed a reporter plasmid, pHBS-TK-CAT, containing two copies of a Hoxb9 binding site (HBSII) upstream from the herpes simplex virus TK promoter, which directs the expression of the CAT gene (Fig. 5A). Transcriptional activity was measured indirectly as the amount of CAT protein production. As shown in Fig. 5B, transfection of HeLa cells with the pHBS-TK-CAT reporter, together with increasing amounts of a construct expressing Hoxb9, activated transcription in a dose-dependent way. The control reporter construct pBLCAT2, lacking the HBSII sites, was not activated when cotransfected with the Hoxb9-expressing vector (Fig. 5B, lane 2). The activity of Hoxb9 was enhanced linearly by cotransfection with increasing amounts of both the Btg1 and the Btg2 expression vectors (Fig. 5C, lanes 3–6). No effect of Btg1 or Btg2 on reporter-gene activity was observed in the absence of Hoxb9 (lanes 8 and 9). To ensure that this increase in transcriptional activity was not a result of additional Hoxb9 production, we monitored protein expression in whole cell extracts using Western blot analysis. As Fig. 5D illustrates, Hoxb9 levels were not increased by Btg1 and Btg2 coexpression. We further looked at the possibility that a control protein could stimulate the transcriptional activation by Hoxb9. Co-transfection of pSG5FlagFOLL, which encodes an unrelated protein, with Hoxb9-expressing vector,
showed no enhancement of the activation (Fig. 5C, lane 7). Next we tested whether other Hox gene products could trans-activate the pHBS-TK-CAT reporter alone or in combination with Btg1 and Btg2. When the reporter construct was cotransfected in HeLa cells with Hoxd9 and Hoxc8 alone, a very weak transactivation was observed. Since we could observe physical interaction in vitro between Hoxc8 and Btg2 (see Fig. 3), we tested if coexpression with Btg proteins could allow Hoxc8 to activate the reporter activity. Expression of Hoxc8 protein, as well as Hoxd9, in combination with Btg1 and Btg2 did not lead to an additional activation (data not shown). Taken together, these results indicate that the HBSII selectively mediate transcriptional activation by Hoxb9 and that its in vivo recognition and activation by a Hoxb9 is essential for the functional effect of the Btg proteins. Thus, it appears that Btg1 and Btg2 expression magnifies the Hoxb9 transcriptional response, which in turn suggests that the Btg proteins can act as effectors of the Hoxb9 signaling pathway.

Identification of the Hoxb9 Activation Domain—Regulation of the activity of transcription factors by other proteins can take place in a number of different ways, one of which involves synergistic effects in which the transcriptional activation caused by two or more transcription factors in combination is much greater than the sum of their individual activities. To test whether the potentiation of the Btg proteins for Hoxb9 activation is due to combinatorial synergism of two activation domains, we first identified the transcriptional activation domain of Hoxb9. For this purpose, we constructed a series of Hoxb9 deletion mutants (Fig. 6A). The transcriptional activity of the N-terminal deletion mutants of Hoxb9, cloned under the control of the SV40 promoter, was examined using the pHBS-TK-CAT reporter plasmid in HeLa cells. As shown in Fig. 6B, transfection of 0.3 μg of the pSGFlagHOXB9 plasmid led to a 13-fold increase in the activation of the pHBS-TK-CAT basal activity. Deletion of the 78 N-terminal amino acids notably decreased the transcriptional activity (Fig. 6B, lane 3). These experiments indicate that the Hoxb9 N-terminal region, extending from amino acids 1 to 78, contains a functional domain that is necessary for transcriptional activation.

Because Btg1 and Btg2 increased Hoxb9-dependent transcription, we next investigated whether the Btg proteins have an intrinsic transcriptional activation domain. We constructed vectors expressing Btg1, Btg2, and various portions of Btg1 cDNA (amino acids 1–38 and 1–96) fused to the Gal4 DNA-binding and dimerization domains of GAL4, downstream from the SV40 early promoter. These constructs were transfected in HeLa cells, together with the pG4-TK-CAT reporter plasmid containing six GAL4 consensus elements upstream from the

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**Fig. 3.** Btg interaction with other Hox proteins: Far Western analysis. GST, purified 6HisBtg2, and induced whole bacterial extract, shown as Coomassie staining, were subjected to 12% SDS-PAGE and transferred from the gel to nitrocellulose.35S-Labeled, in vitro translated, full-length Hoxc8 and Hoxd9 suspended in binding buffer were used as probes as in Fig. 2. Hoxc8 interacted specifically with Btg2 protein.

**Fig. 4.** The effect of Btg2 on DNA binding by Hoxb9 at HBSII site and the formation of a Btg2-Hoxb9-DNA ternary complex, as detected by EMSA. A, sequences of the HBSII and mHBSII oligos. The core binding site is shown in bold letters. B, the 32P-labeled HBSII probe (5 × 106 cpm), containing the binding site for Hoxb9, was incubated with 2.5 μg of GSTHoxb9 alone or in the presence of 2.5 μg of 6HisBtg2 or 6His-Frg. The composition of the binding reactions was as indicated at the top of the EMSA lanes. Binding was competed by 100- and 500-fold molar excess of unlabeled oligonucleotides, as indicated. In lane 11, a 500-fold molar excess of cold HBSII was used.
TK promoter region fused to the CAT gene. No transactivation was detected for any Gal4-Btg1 fusion proteins, even though Gal4VP16 construct exhibited potent activity (data not shown). This indicates that Btg1 and Btg2 lack an intrinsic transactivation domain. Consistent with this observation, neither Btg1 nor Btg2 was able to enhance Hoxb9/79–250-dependent transcription (Fig. 6B, lanes 4 and 5), suggesting that transcriptional stimulation by Btg proteins requires the activation domain of Hoxb9. To further characterize the transcriptional properties of Hoxb9, and its functional interaction with the Btg proteins, we examined its activity when directed to a heterologous DNA binding element through the GAL4 DNA-binding domain. For this purpose, we constructed an expression plasmid that encoded a fusion protein containing the GAL4 DNA-binding domain fused to the full-length HOXB9 cDNA (pGalHOXB9). As reporter plasmid, we used the pG4-TK-CAT plasmid. The chimeric protein Gal4Hoxb9 was unable to activate transcription of pG4-TK-CAT, even in the presence of co-expressed Btg proteins (data not shown). Therefore, Btg proteins do not generically stimulate transcription through the minimal TK promoter, and Hoxb9-Btg functional interaction appears to require the in vivo recognition of the Hoxb9-responsive element.

**DISCUSSION**

Although little is known about the biological functions of Btg proteins, it is likely that they constitute a family of functionally related genes that are involved in the control of the cell cycle. As part of our ongoing research into their biological functions, we used a yeast two-hybrid screening to look for possible functional partners of Btg1 and Btg2. This study describes the physical and functional association between both proteins and Hoxb9. We show that the Btg proteins can modulate the transcriptional activity of Hoxb9 and that the transcriptional activity of the Hoxb9-Btg complex is dependent essentially on the activation domain of Hoxb9, which is located in its N-terminal portion. Together with our previous work, these results support the conclusion that Btg1 and Btg2 may play a role in transcription regulation. In fact, a second protein was isolated by the two-hybrid assay, namely Caf1 (12), the mouse homolog of the yeast yCAF1/POP2 transcription factor.

The functional specificity of the Hoxb9-Btg complex in regulating transcription was tested in transient co-transfection experiments, using a regulatory element, i.e. the promoter for the neural cell-adhesion molecule (N-CAM), which has been well defined in vitro and in vivo (23–27). It contains a 47-bp region, which includes two potential homeodomain binding sites (HBSI and HBSII). HBSII is a target site for the binding and transcriptional control of the N-CAM promoter by the homeoprotein Hoxb9 in vitro and in vivo (23, 25). We show, using the HBSII regulatory element, that the Btg proteins can modulate Hoxb9 transcriptional activity by acting as a transcriptional cofactor. Btg proteins enhance activation of Hoxb9, but not of Hoxc8 or Hoxd9, on the Hoxb9-responsive element, although Hoxc8 is able to interact with Btg2 in vitro (Fig. 3). In addition, Btg proteins were not found to affect the transcriptional activity of Gal4Hoxb9 chimeric protein, indicating that the native conformation of Hoxb9, and its binding to a natural DNA-binding site, are necessary for the assembly of an active Btg-Hoxb9 transcriptional complex. The transcriptional activity of Hoxb9 on HBSII sites was used in the characterization of its functional domains. The main transcriptional activation domain was mapped to the N-terminal region of the Hoxb9 protein, and the deletion of the 78 N-terminal amino acids of Hoxb9 considerably reduced its transcriptional activity. Btg1 and Btg2 enhance Hoxb9-dependent transactivation, and since they do not contain an intrinsic transactivation domain and to date have not been found to bind directly to DNA, they may act as adaptors to promote the interaction between Hoxb9 and the RNA polymerase II basal transcription complex, or to recruit coactivators. Both Btg1 and Btg2 interact with mCaf1, whose yeast homolog is a component of the Ccr4-NOT transcriptional complex, which can affect transcription in either a positive or negative manner (28). Because of the evolutionary conservation of Caf1 among eucaryotes, it is likely that this complex is conserved between yeast and the higher eucaryotes, and that the Btg proteins modulate Hoxb9 transcriptional activity by recruit-
A major question concerning the activity of Hox proteins is how they regulate in vivo transcription with high biological specificity, given that they all contain a highly homologous DNA-binding homeodomain. For a subset of Hox proteins, containing a conserved hexapeptide located N-terminal to the homeodomain, functional specificity would appear to result from interaction with a group of homeodomain-containing proteins, which are Pbx in vertebrates (30–32) and extradenticle (exd) in Drosophila (33, 34, 35). The interaction of Hox proteins with the Exd-Pbx proteins on specific composite DNA sites has been found to enhance Hox-DNA binding affinity, and may account for their functional differences in vivo (30, 36, 37). Interestingly, Hoxb9 does not contain the conserved hexapeptide, or any similar motif, and most likely does not cooperate with Pbx proteins. On the other hand, some recent results have indicated that Hox proteins can bind to a highly overlapping spectrum of monomer binding sites in vivo, as they are already known to do in vitro (39, 40). In this case also, the target-gene specificity of Hox gene products is critically dependent on interaction co-factors that modulate their ability to regulate the transcription of downstream genes. Few Hox cofactor proteins have yet been described, and our results suggest that Btg1 and Btg2 may be two of these factors. Genetic and biochemical studies of Hoxb9 show that it is a multifaceted factor, capable of mediating the transcriptional activation of target genes during mouse embryogenesis, and also in the adult mouse. The destruction of the HOXB9 gene in the mouse causes defects in the development of thoracic skeletal elements (41). In addition to its involvement in embryogenesis, Hoxb9 is involved in the development of the mammary gland in response to pregnancy (42). Clearly, there is no single interaction that is responsible for the different properties of Hoxb9, but different Hox-cofactor complexes may regulate distinct sets of downstream genes. In addition, the discovery of the interaction between Hoxb9 and the Btg proteins increases the number of interaction combinations that can modulate Hoxb9 protein activity. The activity of Hoxb9 during embryogenesis and adult life, and its specific targets, will need to be studied in order for the biological significance of these interactions to be understood. Nevertheless, it is tempting to speculate that the neural cell adhesion molecule gene N-CAM may be the target gene for the Hoxb9-Btg complex. The N-CAM and HOXB9 genes have been found to exhibit collinear expression patterns during spinal cord development (43, 44). Interestingly, the rat Btg2 homolog, PC3, is expressed in the neural tube during neural development (7). In addition, the N-Cam protein is involved in the density-dependent inhibition of growth in mouse fibroblast cell lines (45) and in primary cultures of rat astrocytes (38). This finding, along with the fact that Btg proteins are involved in the negative circuits that govern cell growth suppression, suggests that the antiproliferative effect of the Btg proteins may to some extent result, via their association with Hoxb9, from the activation of the N-CAM gene. In conclusion, our data show Btg proteins to be novel regulators of transcription that lack an intrinsic transactivation domain, but that probably enhance the recruitment of coactivators. Additional work will be needed to define the mechanism by which the Btg proteins enhance Hoxb9 transcription and the functional significance of these associations. The fact that Btg proteins interact with Hoxb9, and probably with other Hox proteins too, as well as with other types of transcription factors, such as mCaf1, suggests that this protein family modulates transcription in response to multiple stimuli.

Acknowledgments—We thank R. Rimokh for providing the pHBS-FlagFOLL plasmid and the 6HisFlag protein, M. Volovitch for Hoxb9- and Hoxc8-expressing vectors, and F. Ruddle for Hox2.5 plasmid.
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