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# CBP and histone deacetylase inhibition enhance the transactivation potential of the HOXB7 homeodomain-containing protein

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**Homeodomain-containing proteins are transcription factors regulating the coordinated expression of multiple target genes involved in development, differentiation and cellular transformation. In this study, we demonstrated that HOXB7, one member of this family, behaved as a transactivator in breast cancer cells. Deletion of either the HOXB7 N-terminal domain or the C-terminal acidic tail abolished this transcriptional effect, suggesting a combination of distinct functional transactivating domains. HOXB7 physically interacted both *in vitro* and *in vivo* with the coactivator CREB-binding protein (CBP). This interaction led to an enhanced transactivating potential and required the N-terminal of HOXB7 as well as two domains located at the C-terminal part of CBP. Moreover, trichostatin A, a deacetylase inhibitor, strongly enhanced the transcriptional properties of HOXB7. Our data therefore indicate that HOX proteins can directly interact with CBP and that acetylation/deacetylation may regulate their transcriptional properties.**

**Keywords:** homeobox gene; CBP; coactivator; transcription; histone acetylation

## Introduction

Genetic programs governing development, differentiation and cell growth imply the regulated transcription of a variety of genes through the action of multiple proteins defined as 'general transcription factors' (GTFs), 'activators', 'repressors' and 'mediators' or 'cofactors', depending on their exact function (Sauer and Tjian, 1997).

The CBP (*C*REB-*b*inding *p*rotein)/p300 proteins are coactivators which interact with the phosphorylated form of CREB (cAMP response element binding) (Chrivia *et al*., 1993) and with a number of other transcription factors including c-*jun*, c-*fos*, nuclear receptors, c-Myb. MyoD, YY1, Sap-1a, sterol regulatory element binding protein (SREBP), E2F1/DP1 (see Shikama *et al*. (1996) for a review), NF-$\kappa$B p65 (Rel A) (Perkins *et al*., 1997), p53 (Avantaggiati *et al*., 1997; Gu and Roeder, 1997a; Lill *et al*., 1997) and Smad proteins (Feng *et al*., 1998; Janknecht *et al*., 1998). All these interactions suggest that CBP acts as a bridge or an 'adaptator' between the activators or repressors and the initiation complex containing the GTFs and the RNA polymerase II (Eckner *et al*., 1994; Arany *et al*., 1995). CBP also interacts with other coactivators such as RAC3 (Li and Don Chen, 1998) and steroid receptor coactivator-1 (SRC-1) (Hanstein *et al*., 1996; Smith *et al*., 1996) and has been copurified with the holoenzyme complex along with BRCA1 (Neish *et al*., 1998). CBP is now considered as an 'integrator' (Kamei *et al*., 1996) or 'co-integrator' (Chakravarti *et al*., 1996) of a variety of signaling pathways. Although both CBP and p300 proteins share similar functional properties in transient transfection experiments, they have distinct functions during retinoic-acid-induced differentiation (Kawasaki *et al*., 1998).

An histone acetyltransferase activity has been attributed to the CBP/p300 proteins (Bannister and Kouzarides, 1996; Ogryzko *et al*., 1996). In other words, CBP can enhance removal of positive charges by acetylation of lysine residues at the N-terminal tail of histones, which presumably destabilizes the nucleosome and facilitates the access of transcription factors to DNA. This histone acetyltransferase activity is required for *in vivo* transcription regulation (Martinez-Balbas *et al*., 1998). CBP also recruits P/CAF, a protein harboring a histone acetyltransferase activity as well (Yang *et al*., 1996). Moreover, CBP/p300 can also acetylate TFIIE, TFIIH (Imhof *et al*., 1997), the erythroid Kruppel-like factor (Zhang and Bieker, 1998), p53 and activate p53 biochemical function (Gu and Roeder, 1997b).

Homeodomain-containing proteins have been identified as transcriptional regulators controlling the expression of genes involved in development, differentiation and tumoral transformation (Levine and Hoey, 1988; Favier and Dollé 1997; Mark *et al*., 1997; Shimamoto *et al*., 1998). They share a highly conserved 60 amino acid DNA-binding domain (the 'homeodomain') (Gehring *et al*., 1994). Although each member of this family exhibits *in vivo* specificity as demonstrated by targeted gene knock-out experiments, they share very similar DNA-binding affinities *in vitro*, suggesting that protein–protein interactions mediate their specificity. For instance, the homeodomain-containing *extradenticle* (exd)/*Pbx* gene products have been identified as cofactors of *HOX* gene products (Mann and Chan, 1996). These interactions require the pentapeptide, a conserved domain located upstream from the DNA-binding domain of most HOX proteins (Chang *et al*., 1995) as well as the Hox cooperativity motif ('HCM'), a sequence carboxy terminal to the Pbx

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homeodomain (Chang *et al*., 1997). Since AbdB-like HOX proteins do not harbor the pentapeptide, most of these products cannot interact with Pbx (Shen *et al*., 1997), thus raising the possibility that other partners might be required. Indeed, a recent report demonstrated the formation of heterodimeric complexes between Hox and Meisl proteins (Shen *et al*., 1997). It is likely that other proteins yet to be identified are involved in such processes.

*HOXB7* was initially isolated from an SV40-transformed human fibroblast cDNA library (Simeone *et al*., 1987) and proposed to be involved in a variety of developmental processes. The HOXB7 protein has also been implicated in hematopoietic differentiation as well as in lymphoid development (Shen *et al*., 1989; Deguchi *et al*., 1991; Lill *et al*., 1995; Magli, 1998). The HOXB7 protein can bind DNA (Corsetti *et al*., 1992) and activate transcription from distinct promoters in a variety of cell lines (Care *et al*., 1996; Chariot *et al*., 1998; Sanlioglu *et al*., 1998). However, the functional domains that mediate HOXB7 transcriptional properties as well as its interacting partners remain to be identified.

In this report, we demonstrated that both the N-terminal domain and the C-terminal acidic tail of HOXB7 are required for transactivation. Moreover, we illustrated the physical interaction between HOXB7 and the coactivator CBP *in vitro* and *in vivo*. We also showed that deacetylase inhibition by trichostatin A (TSA), potentiates the HOXB7 transactivating effect.

## Results

### *A combinatorial code mediates the HOXB7 transactivation effect*

We previously demonstrated that the HOXB7 protein can activate transcription in MDA-MB231 cells (Chariot *et al*., 1998). Indeed, cotransfection of the pTCBS plasmid and the HOXB7 expression vector increased the luciferase activity 3.5- to 3.7-fold over basal activity in a dose-dependent manner (Figure 1b, left panel). A decrease in the transactivating effect was observed when 2 $\mu$g of HOXB7-expression vector was transfected, probably reflecting a squelching effect (Figure 1b, left panel). The commitment of the HOXB7 binding to the CBS sequence was indirectly confirmed by cotransfection of the pT109 reporter plasmid.

To further map the domain(s) that mediate(s) HOXB7 transcriptional activity, expression vectors generating HOXB7 proteins deleted either in the N- or in the C-terminal domain were constructed (Figure 1a). 'B7-ΔN18', 'ΔN54' and 'ΔN86' generated HOXB7 gene products lacking 18, 54 and 86 amino acids in the N-terminal region, respectively. These proteins harbored the pentapeptide domain, whereas the 'B7-ΔN129' construct encoded a HOXB7 gene product lacking the pentapeptide. The product 'B7-ΔC12' lacked 12 amino acid in the C-terminal region and did not contain the acidic tail, whereas the constructs 'B7-ΔC34'. 'B7-ΔC80' and 'B7-ΔC97' were deleted of 34, 80 and 97 amino acids, respectively. Neither 'B7-ΔC80' nor 'B7-ΔC97' products contained the homeodomain. In transient expression experiments, the 'B7-ΔN18' product transactivated (a 2.3- to 2.8-fold induction of luciferase activity) (Figure 1b, middle panel) almost as efficiently as the wild-type HOXB7 protein. A further deletion of 54, 86 or 129 amino acids progressively decreased the transactivating ability of the HOXB7 protein, suggesting that the N-terminal domain of this gene product mediates its transactivating effect. Interestingly, the 'B7-ΔC12' product did not exhibit any transactivation activity and a dose-dependent repressing effect was even observed (Figure 1b, right panel), indicating that the C-terminal acidic

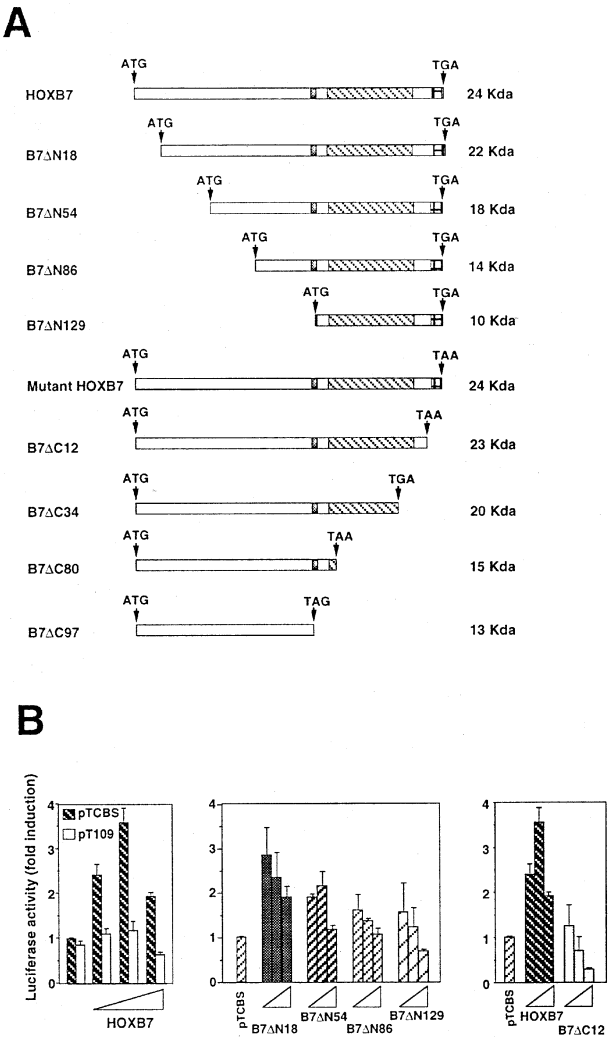


**Figure 1** (**a**) Schematic representation of the HOXB7 expression vectors. These vectors generate products deleted either in their N-terminal domain ('B7-ΔN18', 'ΔN54', 'ΔN86' and 'B7-ΔN129') or in their C-terminal domain ('B7-ΔC12', B7-ΔC34', 'B7-ΔC80' and 'B7-ΔC97'). The homeodomain is illuatrated by a hatched rectangle while both the pentapeptide and the acidic C-terminal tail are also shown. The expected molecular mass of the resulting proteins is given on the right. (**b**) Analysis of the transcriptional properties of HOXB7 wild-type (left panel) and HOXB7 mutant gene products deleted either in their N-terminal domain (middle panel) or in their C-terminal domain (right panel). The pT109 does not contain any HOX-binding sequence and was used as a negative control. Cells were transfected with increasing amounts of HOXB7 wild-type or mutant expression vectors (0.5, 1 or 2 $\mu$g) together with 1 $\mu$g of reporter plasmid. The figure shows the relative luciferase activity over the activity observed with 1 mg of the pTCBS or pT109 reporter plasmids alone. Each value represents the mean ($\pm$s.d.) of at least three independent experiments after normalization to the protein concentration of the extracts

tail is also involved in the HOXB7-dependent transactivation effect. Therefore, we conclude that both the N-terminal domain and the acidic C-terminal tail are required for the HOXB7 transcriptional properties.

### *The HOXB7 protein physically interacts with the coactivator CBP* in vitro

We investigated whether HOXB7 could interact with the coactivator CBP, which is a large and complex protein harboring distinct functional domains (Figure 2a). The GST-CBP fusion proteins (Figure 2b) were expressed in *E.coli*, purified and incubated with *in vitro*-translated HOXB7 protein. HOXB7 was not precipitated by the GST-CBP '1' (Figure 3a, lane 1), a fusion protein harboring both the receptor-interacting domain ('RID') and the CREB binding domain ('KIX') of CBP nor by GST-CBP '2' (Figure 3a, lane 2) which harbors the CREB binding domain ('KIX'), suggesting that there was no physical interaction between HOXB7 and these parts of the CBP protein. However, when HOXB7 was incubated with the GST-CBP '3' fusion protein, a weak signal was detected (Figure 3a, lane 3), suggesting that the histone acetyltransferase domain contributes to the interaction with HOXB7 *in vitro*. Moreover, HOXB7 was clearly precipitated by the GST-CBP '4' fusion protein (Figure 3a. lane 4), suggesting that the cystein-histidine-rich 'C/H3' domain of CBP physically interacts with HOXB7. A signal was also observed when the GST-CBP '5' fusion protein which harbors the C-terminal domain of CBP was incubated with the homeodomain-containing protein (Figure 3a, lane 5). These results suggest that HOXB7 interacts *in vitro* with two domains of CBP known to mediate physical interaction with other transcription factors such as c-*fos*, TFIIB and MyoD for the C/H3 sequence and p53 for the C-terminal domain (Figure 2a).

### *HOXB7 N-terminal domain is required for interaction with CBP*

To further map the HOXB7 domain(s) involved in the physical interaction with CBP. various HOXB7 vectors were used as templates for *in vitro* translation, and translated products were incubated with the GST-CBP '4' fusion protein. As illustrated in Figure 3b, the deletion of the first 18 amino acids clearly abolished HOXB7-CBP interaction (lane 5). Moreover, we could not detect any interaction between CBP and 'B7-ΔN54', 'B7-ΔN86' and 'B7-ΔN129' (Figure 3b, lanes 8, 11 and 14). The N-terminal domain of HOXB7 is thus required for the interaction with CBP *in vitro*.

To determine whether other HOXB7 domains were involved in the interaction with CBP, several HOXB7 vectors deleted in the C-terminal domain were *in vitro* translated. As illustrated in Figure 3c, the mutant B7, which codes for a naturally occurring truncated HOXB7 protein lacking two amino acids within the acidic tail (Chariot *et al*., 1998) and the 'B7-ΔC12' protein interacted with CBP, just as efficiently as the wild-type *HOXB7* gene product (lanes 5 and 8). The 'B7-ΔC34', 'B7-ΔC80' and 'B7-ΔC97' proteins which

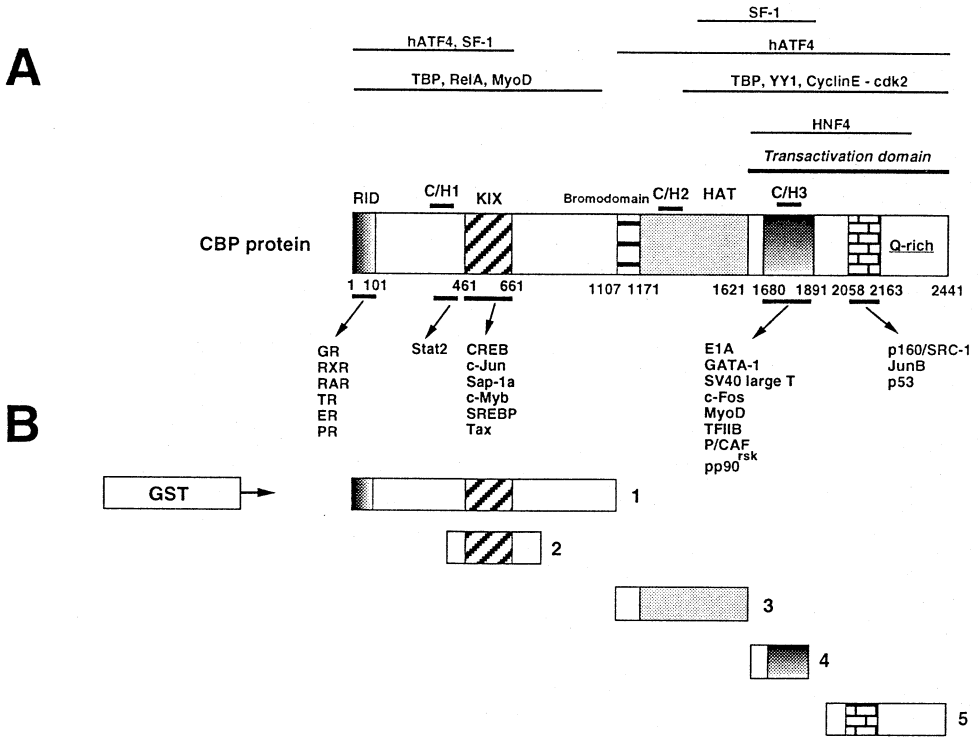


**Figure 2** (**a**) Schematic representation of the CBP coactivator. All the proteins known to physically interact with CBP are mentioned next to their respective interacting CBP domains. 'AR': androgen receptor; 'cdk-2': cyclin-dependent kinase-2; 'C/H3': cystein-histidine-rich domain 3; 'ER': estrogen receptor; 'GR': glucocorticoid receptor; 'HAT': histone acetyltransferase; 'PR': progesterone receptor; 'RID': receptor interacting domain; 'RXR': retinoid X receptor; 'TBP': TATA-binding protein; 'TR': thyroid receptor. (**b**) Schematic representation of the various GST-CBP fusion proteins used in this study

lack the acidic tail and a part or the entirety of the homeodomain could still interact with CBP *in vitro* (Figure 3c, lane 11, 14 and 17). The *in vitro* interaction between HOXB7 and CBP thus requires the N-terminal of this HOX protein.

### *CBP enhances the transactivation potential of HOXB7 in MDA-MB231 cells*

To determine whether HOXB7 and CBP interacted *in vivo*, we performed transient expression experiments in MDA-MB231 cells using either pTCBS or pT109 reporter plasmids, the CMX-CBP expression vector, and HOXB7 expression vectors (Figure 1a). CBP enhanced the HOXB7-mediated transactivation, since a 160-fold induction of luciferase activity was measured when both HOXB7-expression vector and a CMX-CBP expression vector were cotransfected with the pTCBS plasmid (Figure 4a). The luciferase activity observed with the pT109 reporter plasmid was much weaker

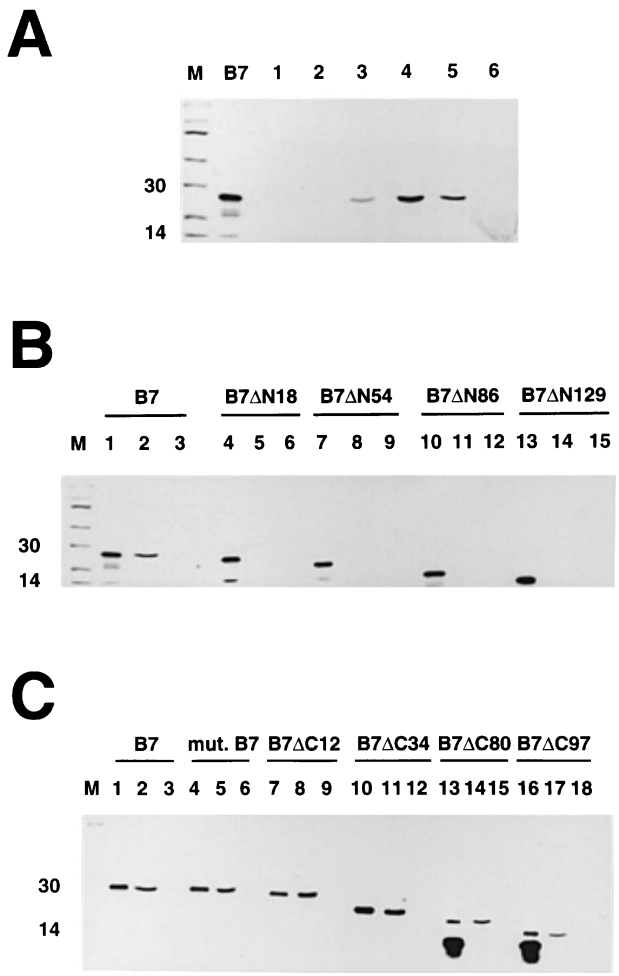


**Figure 3** *In vitro* protein–protein interaction between HOXB7 and CBP. (**a**) The *in vitro* translated wild-type HOXB7 gene product ('B7') was incubated with the GST-CBP'1' (lane 1), GST-CBP'2' (lane 2), GST-CBP'3' (lane 3), GST-CBP'4' (lane 4), GST-CBP'5' (lane 5) or with the GST protein used as a negative control (lane 6). Lane 'B7' represents the *in vitro* translated HOXB7 protein. (**b**) and (**c**): Incubation of the GST-CBP'4' (lanes 2, 5, 8, 11, 14, 17) or the GST protein (lanes 3, 6, 9, 12, 15, 18) with various *in vitro* translated HOXB7 proteins deleted either in their N-terminal (**b**) or in the C-terminal domain (**c**). In each case, 10% of *in vitro* translated material was run as control (lanes 1, 4, 7, 10, 13, 16)

(Figure 4a), demonstrating that the effect is mainly mediated by the binding of HOXB7 to the CBS. We also constructed a CBP mutant named 'CBPΔC' that lacks the HAT, C/H3 and C-terminal domains (Figure 4a). The induction of luciferase activity was much less intense when we cotransfected this CBP mutant with the HOXB7-expression construct and the pTCBS plasmid, thus confirming that these CBP domains are required for the interaction with HOXB7 *in vivo* as well as *in vitro*. Cotransfection of both the 'B7-ΔN129' and CBP expression vectors with the pTCBS reporter plasmid did not significantly enhance the effect of this deleted HOXB7 product (Figure 4b), confirming that the N-terminal domain of HOXB7 is necessary for interaction with the coactivator as shown *in vitro*.

Modulation of HOXB7 transcriptional properties by protein acetylation/deacetylation was investigated by treating MDA-MB231 cells for 20 h with TSA, a deacetylase inhibitor. TSA strongly induced luciferase activity after transfection of both pTCBS and HOXB7 expression vectors (Figure 4c). Interestingly, TSA did not significantly induce the transactivation effect of either the 'B7-ΔN129' product or the 'B7-ΔN18' protein, which still exhibited transactivation abilities (Figure 4c). Taken together, our results suggest that HOXB7 transcriptional properties might be regulated by acetylation/deacetylation through the N-terminal domain of this transcription factor.

We further investigated *in vivo* interaction between CBP and HOXB7 proteins by performing Mammalian two-hybrid system experiments in MDA-MB231 cells using both GAL4-CBP and HOXB7-VP16 expression vectors and the pSG5 reporter plasmid that harbors a GAL4 binding sequence upstream from a minimal E1B promoter and a CAT gene. No significant induction of the CAT activity was measured when the HOXB7-VP16 expression vector was cotransfected with the GAL4 expressing construct and the reporter plasmid, whereas a 2.8-fold induction was observed when both GAL4-CBP and VP16 were expressed, probably because of the intrinsic transactivation ability of the wild-type CBP protein (Figure 5). A sixfold induction of CAT activity was measured when both GAL4-CBP and HOXB7-VP16 expressing constructs were expressed (Figure 5). Moreover, when the HOXB7-VP16 was cotransfected with the 'GAL4-mCBP' construct that generates a GAL4-CBP fusion protein deleted of both the C/H3 and Q-rich domains of CBP, the induction of CAT activity was significantly reduced. These results confirmed that HOXB7 and CBP proteins interact *in vivo* through the C/H3 and Q-rich domain of the coactivator.

## Discussion

In this paper, we have demonstrated that the transactivating effect of HOXB7 in MDA-MB231 cells requires both the N-terminal domain and the C-terminal acidic sequence, thus suggesting a 'combinatorial code' that mediates HOXB7 transcriptional properties. This 'combinatorial code' hypothesis is supported by other studies demonstrating that the transcriptional properties of other HOX proteins are also mediated by a unique combination of several

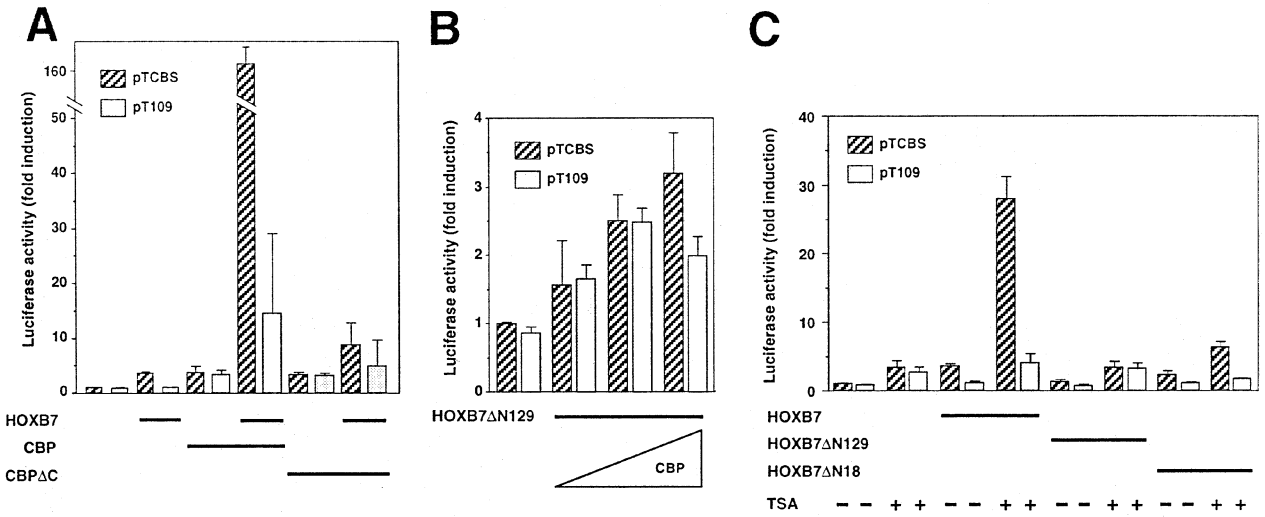


**Figure 4** CBP and a histone deacetylase inhibitor increase HOXB7 transcriptional activity. MDA-MB231 cells were transfected with HOXB7 and/or CBP expression vectors together with the pTCBS (hatched columns) or pT109 (white columns) reporter plasmids. Cellular extracts were prepared and luciferase activities determined. (**a**) Cotransfection with the HOXB7 expression vector (1 $\mu$g) together with expression vectors coding for wild-type (CBP) or mutant (CBP$\Delta$C) protein (2 $\mu$g). (**b**) Cotransfection of the 'B7-$\Delta$N129' expression vector (1 $\mu$g) together with increasing amounts of the CBP expression vector (0.5, 1 or 2 $\mu$g). (**c**) After transfection with the HOXB7 expression vector (1 $\mu$g), the 'B7-$\Delta$N129; or the 'B7-$\Delta$N18' expression vectors (1 $\mu$g), MDA-MB231 cells were left untreated (–) or treated for 20 h with TSA (400 nM) as indicated in the figure

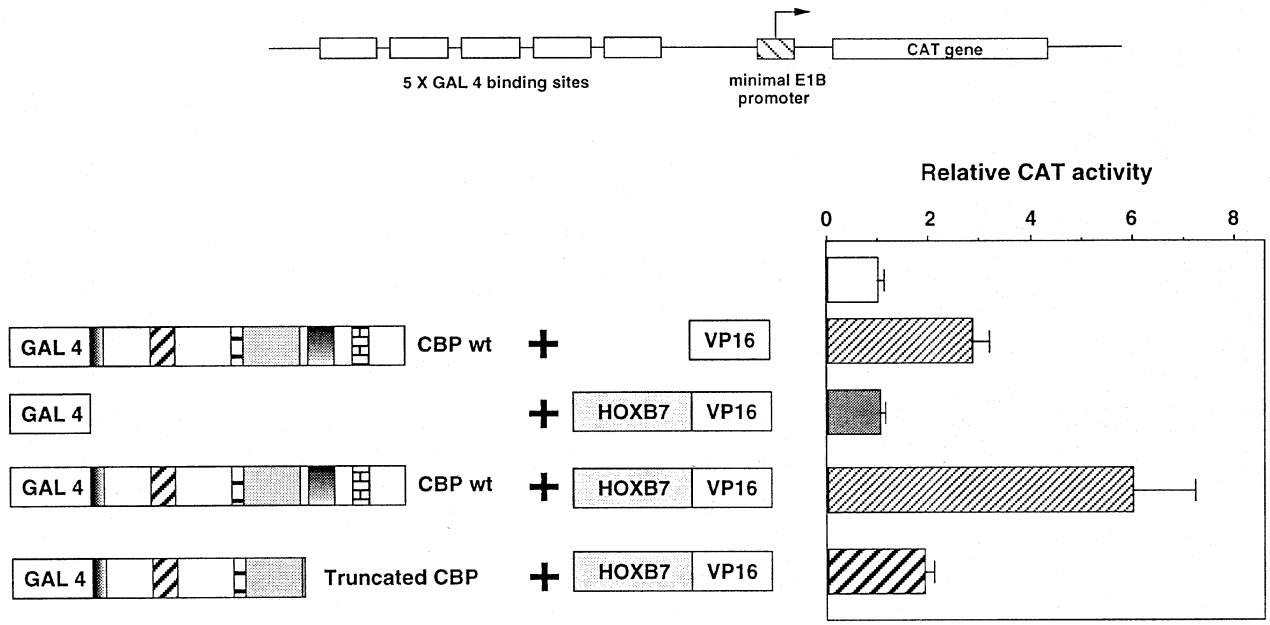


**Figure 5** Interaction between the HOXB7 and CBP proteins by Mammalian two-hybrid system. The pG5 reporter plasmid is schematically represented. All the expression vectors that generate the fusion proteins are illustrated. The MDA-MB231 cells were transfected with 1 $\mu$g of the pG5 and 2 $\mu$g of expression vectors. The figure shows the relative CAT activity over the activity measured with 1 $\mu$g of the pG5 reporter plasmid alone. Each value represents the mean ($\pm$s.d.) of at least three independent experiments after normalization

domains (Schnabel and Abate-Shen, 1996; Zhang *et al*., 1996; Vigano *et al*., 1998).

Despite of the *in vivo* biological specificity of each *HOX* gene product, all these transcription factors share very similar *in vitro* DNA-binding affinities. Protein–protein interactions thus likely play a major role in the regulation of their function. Partners for HOX proteins are, for instance, Pbx and Meis proteins (Mann and Chan, 1996; Shen *et al*., 1997). Since most of these proteins have yet to be identified, we investigated whether the coactivator CBP could modulate the transcriptional properties of HOXB7. Mammalian two-hybrid system experiments, transient transfections and *in vitro* studies indicated that HOXB7 and CBP interact *in vitro* and *in vivo*. Interaction between HOXB7 and CBP requires the HOXB7 N-terminal domain, which is also involved in interactions with other transcription factors such as Meis 1 (Shen *et al*.,

1997). Since this N-terminal domain is less conserved between the various homeodomain-containing proteins, it is tempting to postulate that interaction with CBP contributes to the *in vivo* specificity of HOXB7. However, it is likely that CBP mediates the transcriptional effect of other homeodomain-containing proteins. In this context, a recent study has demonstrated that the pituitary-specific factor Pit-1 requires a coactivator complex that includes both CBP and p/CAF (Xu *et al*., 1998).

TSA enhanced the transactivating effect of the wild-type HOXB7 protein in MDA-MB 231 cells. This deacetylase inhibitor has been previously described as an activator of the gamma globin gene (McCaffrey *et al*., 1997) and the WAF1/Cip1 gene promoter through Sp1 sites (Sowa *et al*., 1997). Moreover, TSA can potentiate retinoid receptor action by altering the chromatin structure within the RAR$\beta$2 promoter (Minucci *et al*., 1997). *In vivo* modification of the chromatin conformation at the HOX-binding sites may then facilitate the access of the homeodomain-containing proteins to regulating sequences. Moreover, our results raise the possibility that HOXB7 function may be regulated by its acetylation, a phenomenon already described for p53 (Gu and Roeder, 1997b), TFIIE, TFIIH (Imhof *et al*., 1997) and the erythroid Kruppel-like factor (Zhang and Bieker, 1998). The acetylation would occur in the N-terminal domain of HOXB7, since we demonstrated that TSA does not significantly enhance the transactivation effect of two *HOXB7* gene products which lack this region. Moreover, deletion of the CBP HAT domain almost completely abolished CBP functional interaction with HOXB7. Alternatively, the acetylation of a cofactor could be required for HOXB7 optimal transcriptional activity. Taken together, these observations suggest that the very strong transcriptional effect observed when HOXB7 and CBP were expressed simultaneously could be due to direct interaction between CBP and HOXB7 that would bridge HOXB7 with the transcription machinery and/or to a reaction of acetylation targeting either HOXB7 itself or a cofactor interacting with HOXB7 N-terminal domain.

The Rubinstein-Taybi syndrome ('RTS') is an autosomal dominant syndrome associated with point mutations in the CBP gene (Petrij *et al*., 1995) and is characterized by craniofacial malformations, broad thumbs, broad big toes and mental retardation. The mutations within the CBP gene lead to the expression of truncated proteins unable to interact with CREB. This hypothesis is supported by a recent study demonstrating a partial similarity to the 'RTS' syndrome in embryos lacking a single *Cbp* allele (Tanaka *et al*., 1997). On the other hand, targeted gene knock-out experiments affecting one or two HOX genes clearly cause developmental malformations (Mark *et al*., 1997). Our data suggest that part of the RTS phenotype might be related to a loss of HOX protein activities during development. Further studies are certainly required to investigate whether a CBP-HOX protein complex is functional during embryogenesis. Moreover, alterations of the CBP/p300 human gene sequences also lead to hematological malignancies (see Giles *et al*., 1998 for a review) whereas a variety of oncogenic translocations involves HOX genes (see Shimamoto *et al*., 1998 for a recent review). Taken together, these observations suggest that both CBP and HOX proteins levels of expression are critical for cellular differentiation and that CBP alteration may contribute to multiple diseases.

## Materials and methods

### *Cell line and treatment*

The MDA-MB231 cell line was obtained from the American Type Tissue Culture Collection (Rockville, MD, USA) and maintained in RPMI medium. MDA-MB231 cells were treated with TSA 400 nM (Sigma, Bornem, Belgium) for 20 h before lysis.

### *Expression plasmids*

Coding sequences of the *HOXB7* gene and of a naturally occuring mutated allele lacking two amino acids in its C-terminal sequence (Chariot *et al*., 1998) were subcloned by polymerase chain reaction (PCR) into the expression vector pcDNA3 (Invitrogen. San Diego, CA, USA). Expression vectors generating truncated *HOXB7* gene products were constructed by PCR (Figure 1a). The constructs 'B7-$\Delta$N18', '$\Delta$N54', '$\Delta$N86' and '$\Delta$N129' generate HOXB7 gene products lacking the 18, 54, 86 and 129 N-terminus amino acids, respectively. The constructs 'B7-$\Delta$C12', 'B7-$\Delta$C34', 'B7-$\Delta$C80' and 'B7-$\Delta$C97' encode HOXB7 proteins lacking the 12, 34, 80 and 97 C-terminal amino acids, respectively. The pT109 and pTCBS reporter plasmids were provided by Dr Zappavigna (Laboratory of Gene Expression, Department of Biology and Technology, Istituto Scientifico H.S. Raffaele, Milan, Italy). The pTCBS plasmid contains an eightfold multimerized form of a homeodomain consensus binding sequence (CBS) cloned upstream from a HSV-TK promoter and of a luciferase (LUC) reporter gene whereas the pT109 construct does not contain the CBS sequence and is used as a negative control (Zappavigna, 1994).

The CMX-CBP expression vector was kindly provided by Dr Evans (The Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA). A functional mouse full-length CBP protein is generated from this vector (Chrivia *et al*., 1993). A CBP mutant named 'CBP$\Delta$C' that lacks the HAT, the C/H3 and the C-terminal domains was amplified by PCR and subcloned in the pcDNA3 vector.

### In vitro *translation*

*In vitro* translations were performed using the 'Wheat germ TNT' kit (Promega, Madison, WI, USA) with 1 $\mu$g of DNA template, 1 $\mu$l of T7 polymerase and [$^{35}$S] methionine. Two $\mu$l of the reaction products were separated by electrophoresis on a 12% polyacrylamide gel followed by autoradiography.

### In vitro *protein–protein interactions*

Plasmids containing fusion genes of GST-CBP '1' (aa 1–1099 of CBP), GST-CBP '3' (aa 1099–1620) and GST-CBP '4' (aa 1620–1877) constructs (Bannister and Kouzarides, 1996) were kindly provided by Dr Kouzarides (Wellcome/CRC Institute, Cambridge, UK) whereas the GST-CBP '2' (aa 390–790) and GST-CBP '5' (aa 1990–2441) plasmids (Gu and Roeder, 1997a) were kindly provided by Dr Roeder (Laboratory of Biochemistry and Molecular Biology, The Rockfeller University, New York, NY, USA).

Expression and purification of GST fusion proteins were performed as described (Kaelin *et al*., 1991) with modifications in the composition of the NENT buffer (NaCl 250 mM, EDTA 1 mM, Tris 20 mM pH 8, NP-40 1.5%). Protein–protein interactions were studied by incubating an aliquot of GST-CBP fusion protein bound to glutathione-Sepharose beads with 10 $\mu$l of *in vitro* translated protein in 200 $\mu$l of TWB buffer (HEPES 20 mM pH 7.9, NaCl 60 mM, dithio-

threitol 1 mM, $MgCl_2$ 6 mM, 8.2% glycerin, EDTA 0.1 mM) for 1 h at 4°C. After six washes of the beads in NENTM buffer, the precipitates were run on an SDS-polyacrylamide gel before autoradiography.

*Transient transfections and luciferase assays*

Transfections in MDA-MB 231 cells were performed using 1 $\mu$g of reporter plasmid and up to 2 $\mu$g of distinct expression vectors per 35-mm dish as described (Chariot *et al*., 1998). Cells were harvested 48 h after transfection and luciferase assays were performed as described (Chariot *et al*., 1998). Transfection efficiency was assessed by transfection of a Luciferase reporter gene driven by the cytomegalovirus (CMV) promoter and by measuring the induction of the LUC activity after cotransfection of the pTCBS reporter plasmid with the HOXB7 expression vector. Luciferase activities were normalized to the protein concentration of the extracts.

*Mammalian two-hybrid system*

The coding sequence of the *HOXB7* gene was subcloned in frame with the activation domain of VP16, whereas a GAL4-CBP expressing construct generating a fusion product containing the coding sequence of CBP in frame with the DNA-binding domain of GAL4 was kindly provided by Dr Evans. A GAL4-CBP construct harboring a stop mutation at amino acid 1630 of the CBP coding sequence and named 'GAL4-mCBP' was kindly provided by Dr Montminy (Laboratory of Advanced Genetic Techniques, Harvard Medical School, Cambridge, MA, USA). Transfection of DNA in MDA-MB 231 cells was performed as described (Chariot *et al*., 1998) and included up to 2 $\mu$g of expressing vectors with 1 $\mu$g of a pG5 reporter plasmid harboring five GAL4 binding sequences upstream from a minimal E1B promoter and a CAT gene (Clontech, Palo Alto, CA, USA). Total amounts of DNA were kept constant by adding appropriate amounts of pcDNA3. Cells were harvested 48 h after transfection and CAT assays were performed as described (Neumann *et al*., 1987).

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