



## COMMENTARY

# The Homeodomain-Containing Proteins

AN UPDATE ON THEIR INTERACTING PARTNERS

Alain Chariot,\* Jacques Gielen, Marie-Paule Merville and Vincent Bours

LABORATORY OF MEDICAL CHEMISTRY AND MEDICAL ONCOLOGY, UNIVERSITY OF LIEGE, LIEGE, BELGIUM

**ABSTRACT.** Homeodomain-containing proteins are transcription regulators controlling the coordinated expression of genes involved in development, differentiation, and cellular transformation. They share a highly conserved 60-amino-acid region (the “homeodomain”), which allows them to bind DNA and modulate the expression of multiple target genes, whose identities remain largely unknown. Although each *HOX* gene product exhibits *in vivo* specificity, they harbor very similar DNA-binding affinities *in vitro*, suggesting that other mechanisms such as protein–protein interactions are critical to modulate their function. In this commentary, we describe the proteins that can interact with the *HOX* gene products, including newly identified partners such as CREB binding protein and the NF- $\kappa$ B/I $\kappa$ B- $\alpha$  proteins. We also outline the molecular programs that are regulated by the transcriptional complexes involving the *HOX* gene products and where new pharmacological tools could find interesting targets. *BIOCHEM PHARMACOL* 58;12:1851–1857, 1999. © 1999 Elsevier Science Inc.

**KEY WORDS.** transcription; homeodomain; *HOX* genes; cofactor; CBP; NF- $\kappa$ B; acetylation

Molecular characterization of the genes whose sequence alterations cause dramatic phenotypes in the fruit fly, *Drosophila melanogaster*, has led to the identification of the *HOX*† genes, defined as “master genes” for the crucial role they play in embryogenesis [1]. They all share a homeobox region, known as a 180-bp highly conserved sequence [2–4] encoding a 60-amino-acid DNA-binding domain (the “homeodomain”) [5], conferring to the resulting proteins the ability to act as transcription factors [6]. The 39 human *HOX* genes are organized in four distinct clusters (loci A, B, C, and D) and can be aligned on the basis of homology within the homeobox to define paralogs [7–10]. Their chromosomal localization within the clusters is closely related to their spatio-temporal pattern of expression along the antero-posterior axis of the developing embryo (“colinearity”) [11]. Beside a critical involvement in cell fate determination, the *HOX* genes also play a key role in differentiation and tumoral development [12, 13].

Extensive expression studies have demonstrated that *Hox* genes harbor overlapping domains of expression in the developing embryos. This observation thus implied that more than one *HOX* protein is expressed in a given cell (“*HOX* code”) [14], and they probably compete for common binding sites within the regulatory sequences of their

target genes. Although each *HOX* protein exhibits *in vivo* specificity, as demonstrated by both targeted gene knockout and gain of function experiments [10, 15], they all share very similar DNA-binding affinities *in vitro* [16, 17]. This paradoxical observation then raised the following questions: how can multiple homeodomain-containing proteins exert their *in vivo* specificity, and what are the rules that govern their ability to activate or repress target genes? In this commentary, we tentatively describe the molecular mechanisms that may resolve these critical issues, and we focus our attention on the mammalian *HOX* proteins.

### IN VITRO AND IN VIVO SPECIFICITIES OF THE *HOX* GENE PRODUCTS: ROLE OF THE COFACTORS

DNA–protein interactions clearly contribute to the *in vivo* specificity of each *HOX* protein [18, 19]. The identification of the functional domains of *HOX* gene products appears to depend on the target DNA sequence but also on the physiological context in which the *HOX* proteins interact with the DNA [20], thus suggesting that interactions with other proteins, defined as “cofactors,” are required to regulate their function.

In the search for *Hox* interacting partners, the first candidates to be identified were the homeodomain-containing PBC proteins, which include the *Drosophila* extradenticle and the vertebrate *Pbx* products [21]. Multiple studies subsequently illustrated the critical role played by the pentapeptide, a conserved domain located upstream from the DNA-binding domain of most Antennapedia-like *HOX* proteins, for the interaction with *PBX* [21–23]. Interestingly, this interaction contributed to the *in vivo*

\* Corresponding author (and present address): Dr. Alain Chariot, Laboratory of Immunoregulation, Room 11B17, Bldg. 10, NIAID, NIH, Rockville Pike, Bethesda, MD 20892, U.S.A. Tel. (301) 496-1664; FAX (301) 402-0070; E-mail: ACHARIOT@niaid.nih.gov

† Abbreviations: AbdB, Abdominal B; CBP, CREB-binding protein; dTCF, *Drosophila* T-cell factor; EKLF, erythroid Kruppel-like factor; *HOX/Hox*, human/murine homeobox gene; Meis, myeloid ecotropic insertion site; NF- $\kappa$ B, nuclear factor  $\kappa$ B; NLS, nuclear localization sequence; *Pbx*, pre-B cell homeobox; Prepl, *Pbx* regulating protein 1; and TSA, trichostatin A.

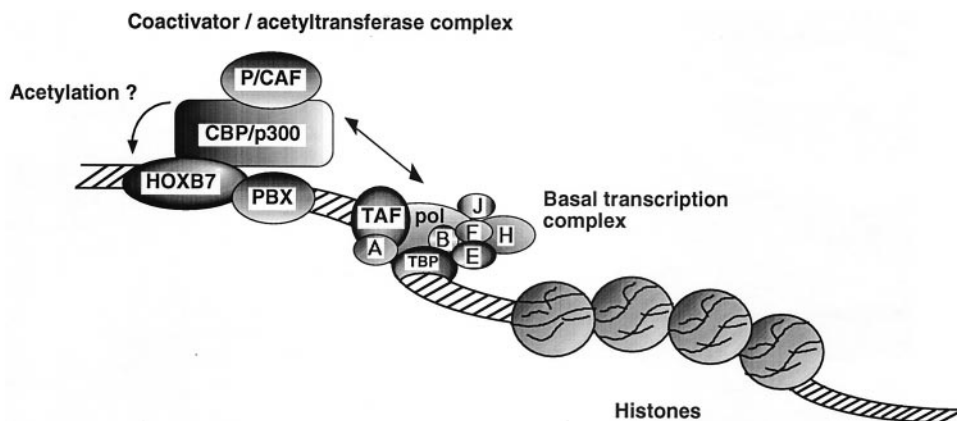


FIG. 1. Schematic illustration of the HOXB7/PBX heterodimer bound to a double-stranded DNA site. The coactivator/acetyltransferase complex composed of CBP/p300 and P/CAF products allowed the homeodomain-containing proteins to access their binding site through acetylation of the histones, represented as four dark circles. Hypothetical acetylation of HOXB7 by CBP is shown. The basal transcription complex, which includes TBP, TFIIA, TFIIB, TFIIE, TFIIIF, TFIIH, TFIIF, the TAFs, and RNA polymerase II, is depicted.

specificity of the HOX proteins [24] through generation of selective HOX/PBX heterodimers bound on a specific bipartite sequence, TGATNNAT [g/t] [g/a] [25–27]. However, it was likely that interactions with additional partners were required, since PBX proteins did not confer DNA binding selectivity on HOX proteins for their target genes [28].

Because most of the AbdB-like HOX proteins do not harbor the conserved pentapeptide, they cannot interact with Pbx [29]. It then was expected that interactions with other partners might regulate their function as well. Indeed, heterodimeric complexes between AbdB-like Hox and Meis1 proteins have been described [30]. In this case, AbdB-like proteins clearly stabilized Meis–DNA interactions. To our knowledge, it is unclear whether functional HOX–Pbx–Meis trimers exist *in vivo*, although such a complex involving HOXA9 has been identified recently *in vitro* and in myeloid cells [31]. On the other hand, stable Meis–Pbx dimers have been identified [32, 33]. Moreover, Prep1, another protein highly related to Meis1, is also a Pbx-interacting partner [34, 35] and is a member of the TALE (“three amino acid loop extension”) family of homeodomain-containing proteins along with Meis and PBC [36]. Interestingly, nuclear localization of PBC proteins might be regulated tightly by their interacting partners, as demonstrated by both biochemical and genetic studies in *Drosophila* [37]. Based on the existence of PBC-containing complexes harboring Hox-independent functions, an attractive hypothesis has been proposed recently [26]. The authors suggest that HOX, instead of PBC proteins, are the real cofactors and may be critical for the regulation of PBC-containing complexes.

#### Histone and Transcription Factor Acetylations: Two Ways to Modulate Hox Protein Functions?

A breakthrough has been made recently regarding the way transcription factors access their target sites, despite a

“repressive” chromatin environment. Indeed, the identification of proteins harboring intrinsic histone acetyltransferase activities allowed major insights into molecular mechanisms of histone acetylation, a phenomenon correlated with transcriptional activity in eukaryotic cells [38]. A histone acetyltransferase (“HAT”) activity has been attributed to several proteins, including CBP/p300 coactivators [39, 40]. Both CBP/p300 proteins were known previously to enhance the transcription potential of a variety of proteins by acting as a bridge or an “adaptor” between the DNA-binding regulators and the basal transcription complex [41–43]. It is now clear that this enhancement of the transcription potential of DNA-binding proteins by CBP/p300 products is mediated by acetylation of lysine residues at the N-terminal tail of histones. This process presumably destabilizes the nucleosome and facilitates the access of transcription factors to DNA.

It was likely that CBP/p300 coactivators enhanced the transcription potential of the homeodomain-containing proteins as well. In this context, Pit-1 transcriptional activity was increased significantly by CBP ([44, 45]; Chariot *et al.*, unpublished data). Moreover, we recently demonstrated a physical interaction between HOXB7, a transactivating protein [46–48], and the coactivator CBP *in vitro* and *in vivo* [49]. This interaction led to an enhanced transactivation by this HOX protein and required the N-terminal of HOXB7 as well as two domains located at the C-terminal part of CBP [49].

Multiple mechanisms that are not mutually exclusive may account for this enhancement of HOXB7 transactivation potential by CBP (Fig. 1). The first mechanism implies CBP as an adaptor or a bridge between HOXB7 bound to its DNA sequence and the basal transcription apparatus, as previously suggested for other transcription factors [41, 42]. The second mechanism refers to a modification of the chromatin conformation within the HOX-binding site due

to the acetylation of the histones either by CBP or by another histone acetyltransferase such as P/CAF, which is also recruited by CBP [50]. The access of the HOXB7 protein to its target sites would then be facilitated. A third mechanism is based on the demonstration that CBP can interact with multiple transcription factors. Then, CBP might bring HOXB7 interacting proteins together with HOXB7, thus creating a strong transactivating complex. A fourth mechanism would involve direct HOXB7 acetylation by CBP. Indeed, we raised the possibility that HOXB7 function may be regulated by its acetylation through its N-terminal domain, since the deacetylase inhibitor TSA increases HOXB7-dependent transcription but does not enhance significantly the transactivation effect of two HOXB7 products lacking this N-terminal region. Interestingly, a lysine residue within this N-terminal domain represents a potential target for acetylation. It then would be of interest to investigate whether HOXB7 is acetylated *in vivo* and whether such post-translational modification is critical for the regulation of its functions. A similar phenomenon has been demonstrated experimentally for a growing number of transcription factors including TFIIE, TFIIF [51], p53 [52], EKLf [53], GATA-1 [54], and dTCF [55]. Therefore, it is believed that acetylation may affect and regulate a variety of substrates involved in distinct pathways.

We now may wonder how interactions of HOX proteins with CBP and/or other coactivators regulate their function *in vivo*. In our experimental system, the HOXB7 N-terminal domain was crucial for the interaction with CBP *in vitro* and in transfected cells. Since this region is less conserved among the HOX proteins, it is tempting to speculate that this interaction contributes to the *in vivo* specificity of the HOX genes. Although not yet experimentally demonstrated, it is likely that CBP also mediates the transcriptional properties of other HOX gene products. It would be of interest to identify the functional domains of the HOX proteins involved in the process. In this context, a recent report demonstrated a functional interaction between CBP and the oncogenic NUP98-HOXA9 fusion protein mediated by the nucleoporin-specific FG repeats [56]. However, the HOXA9 portion of the fusion protein did not co-immunoprecipitate with CBP, whereas the ability of the wild-type HOXA9 protein to interact with this coactivator remains unknown. In any case, the limiting levels of the CBP/p300 proteins within the nucleus may be crucial in selecting the HOX candidates with which to interact. Indeed, competition between distinct families of transcription factors for interaction with CBP can influence their function dramatically [57]. Therefore, it will be interesting to define the parameters that govern the ability of multiple HOX proteins to interact preferentially with limiting levels of CBP and/or other coactivators within a single cell and to determine whether these parameters can be integrated to a whole organism.

### **Modulation of Hox Protein Function by Phosphorylation**

It has been known for many years that post-translational modifications of transcription factors can modulate their function materially. Among these processes, phosphorylation/dephosphorylation is the most widely described regulating process [58]. Due to many putative phosphorylation sites and to the elevated number of HOX genes, the modulation of HOX protein functions by phosphorylation still remains largely unexplored. However, few reports have attempted to address this issue. The *Drosophila* Antennapedia protein was phosphorylated by casein kinase II at critical residues [59]. An Antennapedia mutant where the phosphorylated residue is replaced by alanine failed to interact with extradenticle, thus causing severe defects in both thoracic and abdominal development [59]. Interestingly, this hypothesis can be extended to other homeodomain-containing proteins that are not encoded by the clustered HOX genes ("divergent HOX proteins"). Indeed, a physical interaction between HOX11 and phosphatases PP2A and PP1 has been reported [60]. Moreover, this process disrupts a G<sub>2</sub>/M cell-cycle checkpoint. These observations emphasize the crucial role played by phosphorylation/dephosphorylation events in the regulation of HOX proteins. They also suggest that post-translational modification of HOX proteins can affect the cell cycle and modulate complex molecular programs involved in cell proliferation and differentiation. These results also raise a number of questions. Which HOX proteins are preferentially phosphorylated? Are the putative HOX protein kinases/phosphatases spatio-temporally regulated in the developing embryo and in differentiated organs? Future investigations undoubtedly will provide further insights regarding these key issues.

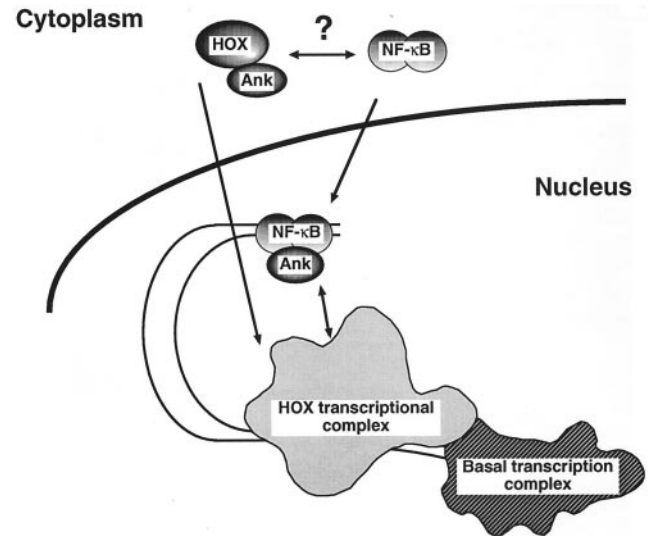
### **Homeodomain-Containing Proteins and NF- $\kappa$ B Members: Evidence for Cross-Talk?**

To study further the transcriptional effects of HOX proteins, we recently demonstrated that HOXB7 interacts with ubiquitously expressed transcription factors belonging to other families. Indeed, using transient expression experiments, we provided evidence that the HOXB7 transactivating potential was increased markedly by NF- $\kappa$ B proteins [61]. These latter transcription factors play a central role in the cellular defense against stress, cytokines, and pathogens [62–64] and regulate a wide spectrum of immune and inflammatory responses in multiple species [65]. Because it is believed that transcriptional synergism between distinct proteins contributes to the regulation of their function, presumably through protein–protein interactions, it was expected that HOX proteins and NF- $\kappa$ B could be involved in such a phenomenon ("cross-talk").

Surprisingly, HOXB7 transcription potential was increased further by I $\kappa$ B- $\alpha$ , the first identified inhibitor of NF- $\kappa$ B activity [66, 67]. The N-terminal domain of HOXB7 and both the ankyrin repeats and the C-terminal

region of I $\kappa$ B- $\alpha$  were required for the physical interaction. Hypothetical models that may account for this enhancement of HOXB7 transactivation potential by I $\kappa$ B- $\alpha$  were proposed [61]. Besides indirect mechanisms, a stabilization of HOXB7 DNA binding by I $\kappa$ B- $\alpha$  ankyrin repeats may represent a first pathway. Another mechanism implies the establishment of HOXB7/NF- $\kappa$ B/I $\kappa$ B- $\alpha$  transcriptional complexes whose respective transactivation domains contribute to the enhanced effect. Further experimental evidence is needed to investigate whether such hypotheses are relevant. The answer, however, may come from an additional mechanism related to the regulation of nuclear localization of HOX proteins by their interacting partners, such as I $\kappa$ B- $\alpha$ . I $\kappa$ B- $\alpha$  initially was described as a cytoplasmic protein [67]; since then, nuclear localization of I $\kappa$ B- $\alpha$  has been demonstrated in a variety of cells [68, 69]. Once in the nucleus, I $\kappa$ B- $\alpha$  can remove NF- $\kappa$ B dimers from functional preinitiation complexes and from their  $\kappa$ B DNA sequences, thus inhibiting NF- $\kappa$ B activity [70]. The way I $\kappa$ B- $\alpha$  enters into the nucleus has been described recently, and involves its second ankyrin repeat acting as a newly described nuclear import sequence [71]. I $\kappa$ B- $\alpha$  nuclear localization also may imply another additional protein(s) able to interact with ankyrin repeats [72]. This latter study, combined with our results, suggests that HOXB7 may be a candidate. The interaction between HOXB7 and I $\kappa$ B- $\alpha$  occurred in the absence of DNA, a result that supports this hypothesis. We also have observed an enhancement of HOXB7 transcription potential by Bcl3, another member of the I $\kappa$ B family, which also harbors ankyrin repeats and a potential NLS (Chariot *et al.*, unpublished results). To date, it is not known whether I $\kappa$ B- $\beta$ , whose putative NLS still has to be identified, can also modulate HOXB7 transcription potential. Anyway, it is tempting to speculate that homeodomain-containing proteins, including HOX gene products, may represent a family of transcription factors that are implicated in the regulation of the nuclear localization of other proteins, such as ankyrin repeat-containing products (Fig. 2). This protein-protein interaction would allow a mutual regulation of their nuclear localization. In such a model, further investigation should determine whether the NLS is provided either by I $\kappa$ B- $\alpha$  or by HOXB7 for the nuclear import pathway and whether the NF- $\kappa$ B heterodimer interferes with this process.

This potential "cross-talk" between NF- $\kappa$ B/I $\kappa$ B- $\alpha$  proteins and HOXB7 is interesting because of the distinct physiological processes that require these transcription factors. Both families of proteins are expressed simultaneously in the developing embryo. Indeed, although the involvement of NF- $\kappa$ B/I $\kappa$ B proteins during development is unclear, NF- $\kappa$ B gene expression has been detected during limb morphogenesis, and alteration of the activity of these proteins causes an arrest of the outgrowth [73, 74]. Moreover, I $\kappa$ B- $\alpha$  is the human homologue of cactus, a protein that plays a crucial role in the dorso-ventral patterning of the *Drosophila* embryo [75]. Since HOX genes clearly are required to establish the antero-posterior axis of the devel-



**FIG. 2.** Mutual regulation of HOXB7/I $\kappa$ B- $\alpha$  nuclear localization through direct protein-protein interaction. A heterodimer that includes an ankyrin repeat-containing protein ("Ank") and a HOX protein ("HOX") moves to the nucleus and subsequently regulates the expression of a HOX target gene. The ankyrin repeat-containing protein, bound to the NF- $\kappa$ B heterodimer in the nucleus, also physically interacts with a HOX transcriptional complex, which includes HOX proteins and the co-activator/acyltransferase complex. These physical interactions contribute to the regulation of the transcriptional ability of the HOX protein. The basal transcription machinery also is illustrated.

oping embryo, it would be interesting to determine whether mutual regulation between I $\kappa$ B- $\alpha$  and HOX proteins occurs during the development of both the antero-posterior and the dorso-ventral axes of the embryo.

#### *Insights into Hox Proteins and Their Interacting Partners: A Way to Design Pharmacological Treatments?*

The critical role played by HOX genes in embryogenesis has been demonstrated by phenotypic abnormalities caused by gene disruption as well as by inherited alterations of limb development due to naturally occurring alterations of HOX genes [76, 77]. Moreover, the involvement of HOX genes in hematopoiesis also has been demonstrated based on extensive expression studies and on the leukemogenic effect of altered HOX genes [78, 79]. On the other hand, the genes coding for most of the HOX interacting proteins have been identified through molecular characterization of rearrangements found in leukemia. Taken together, these observations suggest that a variety of diseases are due to the disruption of protein-protein interactions involving HOX proteins, leading to the subsequent dysregulation of the HOX target genes. Moreover, the genetic programs controlled by the HOX proteins also regulate physiological functions [80, 81]. A better understanding of these protein functions and the mechanisms regulating their activity, including protein-protein interactions, might allow the development of novel therapies that could specifically restore and/or affect HOX-dependent regulating pathways.

## CONCLUSIONS

Significant progress has been made recently regarding the molecular characterization of the transcriptional complexes involving *HOX* gene products. The aim of this commentary was to demonstrate tentatively that elucidation of genetic programs regulated by *HOX* genes can be achieved through identification of *HOX* interacting partners. Although it is likely that most of the actors still have to be defined, it is clear that further investigations regarding the way the known *HOX* partners interact with each other will lead to a better understanding of the crucial role played by *HOX* genes in development, differentiation, and disease.

---

*The authors are grateful to Carine van Lint for helpful discussions and to Ulrich Siebenlist for his support. Alain Chariot is a Research Assistant at the University of Liege and is supported by postdoctoral grants from the NATO and the Fulbright Commission. Vincent Bours and Marie-Paule Merville are Research Associates of the National Fund for Scientific Research ("FNRS," Belgium).*

---

## References

- McGinnis W and Krumlauf R, Homeobox genes and axial patterning. *Cell* **68**: 283–302, 1992.
- McGinnis W, Levine MS, Hafen E, Kuroiwa A and Gehring WJ, A conserved DNA sequence in homeotic genes of the *Drosophila* Antennapedia and bithorax complexes. *Nature* **308**: 428–433, 1984.
- Levine M, Rubin GM and Tjian R, Human DNA sequences homologous to a protein coding region conserved between homeotic genes of *Drosophila*. *Cell* **38**: 667–673, 1984.
- Scott MP and Weiner AJ, Structural relationships among genes that control development: Sequence homology between the *Antennapedia*, *Ultrabithorax*, and *fushi tarazu* loci of *Drosophila*. *Proc Natl Acad Sci USA* **81**: 4115–4119, 1984.
- Gehring WJ, Affolter M and Bürglin T, Homeodomain proteins. *Annu Rev Biochem* **63**: 487–526, 1994.
- Levine M and Hoey T, Homeobox proteins as sequence-specific transcription factors. *Cell* **55**: 537–540, 1988.
- Acampora D, D'Esposito M, Faiella A, Pannese M, Migliaccio E, Morelli F, Stornaiuolo A, Nigro V, Simeone A and Boncinelli E, The human *HOX* gene family. *Nucleic Acids Res* **17**: 10385–10402, 1989.
- Scott MP, Vertebrate homeobox gene nomenclature. *Cell* **71**: 551–553, 1992.
- Duboule D, *Guidebook to the Homeobox Genes*. Oxford University Press, Oxford, 1994.
- Maconochie M, Nonchev S, Morrison A and Krumlauf R, Paralogous *HOX* genes: Function and regulation. *Annu Rev Genet* **30**: 529–556, 1996.
- Graham A, Papalopulu N and Krumlauf R, The murine and *Drosophila* homeobox gene complexes have common features of organization and expression. *Cell* **57**: 367–378, 1989.
- Favier B and Dollé P, Developmental functions of mammalian *Hox* genes. *Mol Hum Reprod* **3**: 115–131, 1997.
- Shimamoto T, Ohyashiki K, Toyama K and Takeshita K, Homeobox genes in hematopoiesis and leukemogenesis. *Int J Hematol* **76**: 339–350, 1998.
- Kessel M and Gruss P, Homeotic transformation of murine prevertebrae and concomitant alteration of *Hox* codes induced by retinoic acid. *Cell* **67**: 89–104, 1991.
- Krumlauf R, *Hox* genes in vertebrate development. *Cell* **78**: 191–201, 1994.
- Kalionis B and O'Farrell PH, A universal target sequence is bound *in vitro* by diverse homeodomains. *Mech Dev* **43**: 57–70, 1993.
- Shang Z, Ebright YW, Iler N, Pendergrast PS, Echelard Y, McMahon AP, Ebright RH and Abate C, DNA affinity cleaving analysis of homeodomain-DNA interaction: Identification of homeodomain consensus sites in genomic DNA. *Proc Natl Acad Sci USA* **91**: 118–122, 1994.
- Dessain S, Gross CT, Kuziora MA and McGinnis W, Antp-type homeodomains have distinct DNA binding specificities that correlate with their different regulatory functions in embryos. *EMBO J* **11**: 991–1002, 1992.
- Pellerin I, Schnabel C, Catron KM and Abate C, Hox proteins have different affinities for a consensus DNA site that correlate with the positions of their genes on the *hox* cluster. *Mol Cell Biol* **14**: 4532–4545, 1994.
- Vigano MA, Di Rocco G, Zappavigna V and Mavilio F, Definition of the transcriptional activation domains of three human *HOX* proteins depends on the DNA-binding context. *Mol Cell Biol* **18**: 6201–6212, 1998.
- Chang C-P, Shen W-F, Rozenfeld S, Lawrence HJ, Largman C and Cleary ML, Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins. *Genes Dev* **9**: 663–674, 1995.
- Knoepfler PS and Kamps M, The pentapeptide motif of Hox proteins is required for cooperative DNA binding with Pbx1, physically contacts Pbx1, and enhances DNA binding by Pbx1. *Mol Cell Biol* **15**: 5811–5819, 1995.
- Phelan ML, Rambaldi I and Featherstone MS, Cooperative interactions between *HOX* and *PBX* proteins mediated by a conserved peptide motif. *Mol Cell Biol* **15**: 3989–3997, 1995.
- Mann RS and Chan S-K, Extra specificity from *extradenticle*: The partnership between *HOX* and *PBX/EXD* homeodomain proteins. *Trends Genet* **12**: 258–262, 1996.
- Di Rocco G, Mavilio F and Zappavigna V, Functional dissection of a transcriptionally active target-specific Hox-Pbx complex. *EMBO J* **16**: 3644–3654, 1997.
- Mann RS and Affolter M, Hox proteins meet more partners. *Curr Opin Genet Dev* **8**: 423–429, 1998.
- Piper DE, Batchelor AH, Chang C-P, Cleary ML and Wolberger C, Structure of a HoxB1-Pbx1 heterodimer bound to DNA: Role of the hexapeptide and a fourth homeodomain helix in complex formation. *Cell* **96**: 587–597, 1999.
- Neuteboom STC and Murre C, Pbx raises the DNA binding specificity but not the selectivity of *Antennapedia* Hox proteins. *Mol Cell Biol* **17**: 4696–4706, 1997.
- Chang C-P, Brocchieri L, Shen W-F, Largman C and Cleary ML, Pbx modulation of Hox homeodomain amino-terminal arms establishes different DNA-binding specificities across the *Hox* locus. *Mol Cell Biol* **16**: 1734–1745, 1996.
- Shen W-F, Montgomery JC, Rozenfeld S, Moskow JJ, Lawrence HJ, Buchberg AM and Largman C, AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. *Mol Cell Biol* **17**: 6448–6458, 1997.
- Shen W-F, Rozenfeld S, Kwong A, Kömüves LG, Lawrence HJ and Largman C, *HOXA9* forms triple complexes with *PBX2* and *MEIS1* in myeloid cells. *Mol Cell Biol* **19**: 3051–3061, 1999.
- Chang C-P, Jacobs Y, Nakamura T, Jenkins NA, Copeland NG and Cleary ML, Meis proteins are major *in vivo* DNA binding partners for wild-type but not chimeric Pbx proteins. *Mol Cell Biol* **17**: 5679–5687, 1997.
- Knoepfler P, Calvo KR, Chen H, Antonarakis SE and Kamps MP, Meis1 and pKnox1 bind DNA cooperatively with Pbx1 utilizing an interaction surface disrupted in oncoprotein E2a-Pbx1. *Proc Natl Acad Sci USA* **94**: 14553–14558, 1997.
- Berthelsen J, Zappavigna V, Mavilio F and Blasi F, Prep1, a

- novel functional partner of Pbx proteins. *EMBO J* **17**: 1423–1433, 1998.
35. Goudet G, Delhalle S, Biemar F, Martial JA and Peers B, Functional and cooperative interactions between the homeodomain PDX1, Pbx, and Prep1 factors on the somatostatin promoter. *J Biol Chem* **274**: 4067–4073, 1999.
  36. Bürglin TR, Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res* **25**: 4173–4180, 1997.
  37. Rieckhof GE, Casares F, Ryoo HD, Abu-Shaar M and Mann RS, Nuclear translocation of Extradenticle requires *homothorax*, which encodes an Extradenticle-related homeodomain protein. *Cell* **91**: 171–183, 1997.
  38. Struhl K, Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* **12**: 599–606, 1998.
  39. Bannister AJ and Kouzarides T, The CBP co-activator is a histone acetyltransferase. *Nature* **384**: 641–643, 1996.
  40. Ogryzko VV, Schiltz RL, Russanova V, Howard BH and Nakatani Y, The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**: 953–959, 1996.
  41. Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB and Livingston DM, Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. *Genes Dev* **8**: 869–884, 1994.
  42. Arany Z, Newsome D, Oldread E, Livingston DM and Eckner R, A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. *Nature* **374**: 81–84, 1995.
  43. Shikama N, Lyon J and La Thangue NB, The p300/CBP family: Integrating signals with transcription factors and chromatin. *Trends Cell Biol* **7**: 230–236, 1997.
  44. Xu L, Lavinsky RM, Dasen JS, Flynn SE, McInerney EM, Mullen TM, Heinzel T, Szeto D, Korzus E, Kurokawa R, Aggarwal AK, Rose DW, Glass CK and Rosenfeld MG, Signal-specific co-activator domain requirements for Pit-1 activation. *Nature* **395**: 301–306, 1998.
  45. Zanger K, Cohen LE, Hashimoto K, Radovick S and Wondisford FE, A novel mechanism for cyclic adenosine 3',5'-monophosphate regulation of gene expression by CREB-binding protein. *Mol Endocrinol* **13**: 268–275, 1999.
  46. Carè A, Silvani A, Meccia E, Mattia G, Stoppacciaro A, Parmiani G, Peschle C and Colombo MP, HOXB7 constitutively activates basic fibroblast growth factor in melanomas. *Mol Cell Biol* **16**: 4842–4851, 1996.
  47. Chariot A, Castronovo V, Le P, Gillet C, Sobel ME and Gielen J, Cloning and expression of a new HOXC6 transcript encoding a repressing protein. *Biochem J* **319**: 91–97, 1996.
  48. Chariot A, Senterre-Lesenfants S, Sobel ME and Castronovo V, Molecular cloning of a mutated HOXB7 cDNA encoding a truncated transactivating homeodomain-containing protein. *J Cell Biochem* **71**: 46–54, 1998.
  49. Chariot A, van Lint C, Chapelier M, Gielen J, Merville M-P and Bours V, CBP and histone deacetylase inhibition enhance the transactivation potential of the HOXB7 homeodomain-containing protein. *Oncogene* **18**: 4007–4014, 1999.
  50. Yang XJ, Ogryzko VV, Nishikawa JI, Howard BH and Nakatani Y, A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382**: 319–324, 1996.
  51. Imhof A, Yang XJ, Ogryzko VV, Nakatani Y, Wolffe AP and Ge H, Acetylation of general transcription factors by histone acetyltransferases. *Curr Biol* **7**: 689–692, 1997.
  52. Gu W and Roeder RG, Activation of p53 sequence specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**: 595–606, 1997.
  53. Zhang W and Bieker JJ, Acetylation and modulation of erythroid Kruppel-like factor (ELKF) activity by interaction with histone acetyltransferases. *Proc Natl Acad Sci USA* **95**: 9855–9860, 1998.
  54. Boyes J, Byfield P, Nakatani Y and Ogryzko V, Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* **396**: 594–598, 1998.
  55. Waltzer L and Bienz M, *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* **395**: 521–525, 1998.
  56. Kasper LH, Brindle PK, Schnabel CA, Pritchard CEJ, Cleary ML and van Deursen JMA, CREB binding protein interacts with nucleoporin-specific FG repeats that activate transcription and mediate NUP98-HOXA9 oncogenicity. *Mol Cell Biol* **19**: 764–776, 1999.
  57. Kamei Y, Xu L, Heinzel T, Torchia J, Kurowaka R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK and Rosenfeld MG, A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**: 403–414, 1996.
  58. Hunter T and Karin M, The regulation of transcription by phosphorylation. *Cell* **70**: 883–894, 1992.
  59. Jaffe L, Ryoo HD and Mann RS, A role for phosphorylation by casein kinase II in modulating Antennapedia activity in *Drosophila*. *Genes Dev* **11**: 1327–1340, 1997.
  60. Kawabe T, Muslin AJ and Korsmeyer SJ, HOX11 interacts with protein phosphatases PP2A and PP1 and disrupts a G2/M cell-cycle checkpoint. *Nature* **385**: 454–458, 1997.
  61. Chariot A, Princen F, Gielen J, Merville M-P, Franzoso G, Brown K, Siebenlist U and Bours V, I $\kappa$ B- $\alpha$  enhances transactivation by the HOXB7 homeodomain-containing protein. *J Biol Chem* **274**: 5318–5325, 1999.
  62. Siebenlist U, Franzoso G and Brown K, Structure, regulation and function of NF- $\kappa$ B. *Annu Rev Cell Biol* **10**: 405–455, 1994.
  63. Baldwin A, The NF- $\kappa$ B and I $\kappa$ B proteins: New discoveries and insights. *Annu Rev Immunol* **14**: 649–681, 1996.
  64. Ghosh S, May MJ and Kopp EB, NF- $\kappa$ B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* **16**: 225–260, 1998.
  65. Baeuerle PA and Henkel T, Function and activation of NF- $\kappa$ B in the immune system. *Annu Rev Immunol* **12**: 141–179, 1994.
  66. Baeuerle PA and Baltimore D, I $\kappa$ B: A specific inhibitor of the NF- $\kappa$ B transcription factor. *Science* **242**: 540–546, 1988.
  67. Haskill S, Beg AA, Tompkins SM, Morris JS, Yurochko AD, Sampson-Johannes A, Mondal K, Ralph P and Baldwin AS Jr, Characterization of an immediate-early gene induced in adherent monocytes that encodes I $\kappa$ B-like activity. *Cell* **65**: 1281–1289, 1991.
  68. Cressman DE and Taub R, I $\kappa$ B $\alpha$  can localize in the nucleus but shows no direct transactivation potential. *Oncogene* **8**: 2567–2573, 1993.
  69. Zabel U, Henkel T, Silva MDS and Baeuerle P, Nuclear uptake control of NF- $\kappa$ B by MAD-3, an I $\kappa$ B protein present in the nucleus. *EMBO J* **12**: 201–211, 1993.
  70. Arenzana-Seisdedos F, Thompson J, Rodriguez MS, Bachevalerie F, Thomas D and Hay RT, Inducible nuclear expression of newly synthesized I $\kappa$ B $\alpha$  negatively regulates DNA-binding and transcriptional activities of NF- $\kappa$ B. *Mol Cell Biol* **15**: 2689–2696, 1995.
  71. Sachdev S, Hoffmann A and Hannink M, Nuclear localization of I $\kappa$ B- $\alpha$  is mediated by the second ankyrin repeat: The I $\kappa$ B- $\alpha$  ankyrin repeats define a novel class of cis-acting nuclear import sequences. *Mol Cell Biol* **18**: 2524–2534, 1998.
  72. Turpin P, Hay RT and Dargemont C, Characterization of I $\kappa$ B $\alpha$  nuclear import pathway. *J Biol Chem* **274**: 6804–6812, 1999.
  73. Bushdid PB, Brantley DM, Yull FE, Blaeuer GL, Hoffman LH, Niswander L and Kerr LD, Inhibition of NF- $\kappa$ B activity

- results in disruption of the apical ectodermal ridge and aberrant limb morphogenesis. *Nature* **392**: 615–618, 1998.
74. Kanegae Y, Tavares AT, Izpisua Belmonte JC and Verma IM, Role of Rel/NF- $\kappa$ B transcription factors during the outgrowth of the vertebrate limb. *Nature* **392**: 611–614, 1998.
  75. Geisler R, Bergmann A, Hiromi Y and Nüsslein-Volhard C, *cactus*, a gene involved in dorsoventral pattern formation of *Drosophila*, is related to the I $\kappa$ B gene family of vertebrates. *Cell* **71**: 613–621, 1992.
  76. Akarsu AN, Stoilov I, Yilmaz E, Sayli BS and Sarfarazi M, Genomic structure of *HOXD13* gene: A nine polyalanine duplication causes synpolydactyly in two unrelated families. *Hum Mol Genet* **5**: 945–952, 1996.
  77. Muragaki Y, Mundlos S, Upton J and Olsen BR, Altered growth and branching patterns in synpolydactyly caused by mutations in *HOXD13*. *Science* **272**: 548–551, 1996.
  78. Borrow J, Shearman AM, Stanton VP Jr, Becher R, Collins T, Williams AJ, Dubé I, Katz F, Kwong YL, Morris C, Ohyashiki K, Toyama K, Rowley J and Housman DE, The t(7;11)(p15; p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin *NUP98* and class I homeoprotein *HOXA9*. *Nat Genet* **12**: 159–167, 1996.
  79. Nakamura T, Largaespada DA, Lee MP, Johnson LA, Ohyashiki K, Toyama K, Chen SJ, Willman CL, Chen I-M, Feinberg AP, Jenkins NA, Copeland NG and Shaughnessy JD Jr, Fusion of the nucleoporin gene *NUP98* to *HOXA9* by the chromosome translocation t(7;11)(p15; p15) in human myeloid leukaemia. *Nat Genet* **12**: 154–158, 1996.
  80. Chen F and Capecchi MR, Paralogous mouse *Hox* genes, *Hoxa9*, *Hoxb9* and *Hoxd9*, function together to control development of the mammary gland in response to pregnancy. *Proc Natl Acad Sci USA* **96**: 541–546, 1999.
  81. Duboule D, No milk today (my Hox have gone away). *Proc Natl Acad Sci USA* **96**: 322–323, 1999.