Minireview

The double life of *HOXB4*

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Abstract ***HOXB4*** **is a homeodomain-containing transcription factor with diverse roles in embryonic development and the regulation of adult stem cells. Intriguingly, this gene can act in opposite ways when expressed by different cells, promoting the proliferation of stem cells whilst activating the apoptotic pathway in some embryonic structures. This review considers the basis for these differences in terms of the molecular biology of** ***HOXB4*** **and the cells that express it.**

Keywords: *HOXB4*; *HOX* gene; Development; Stem cell; Haemopoiesis; Apoptosis

1. Introduction

One of the key events in development and in the renewal of adult issues is the specification and maintenance of cellular identity. The genes that underlie this often consist of closely related groups that are frequently conserved across different animal phyla. Here, we consider one of the best known and most extensively studied examples, the *HOX* genes. These are a family of transcription factors characterised by a very highly conserved DNA-binding domain, and which are clustered in closely linked groups on chromosomes, their order within these groups being reflected in both the timing and region of expression in the embryo. Mutation or substitution of a *HOX* gene can lead to the replacement of one body part by another, reflecting the key significance of these genes in determining cellular identity [1–4]. In addition to numerous functions during development, the *HOX* genes later regulate stem cell proliferation and differentiation, in what is arguably an extension of embryogenesis into the adult form.

The ability of *HOX* genes to determine cellular identity may lie in part with their unusual property of both repressing and activating the transcription of different target genes. Positive regulation by HOX proteins requires the binding of a number of co-factors, most notably members of the PBX and MEIS transcription factor families [5–12]. This interaction increases the binding specificity of HOX proteins for specific target sequences in the enhancer and promoter regions of target genes. A recent study has suggested that *PBX* and *MEIS* may also assist in *HOX* target activation by penetrating inactive chromatin, thereby allowing access to previously hidden sites [13].

HOX mediated repression may occur by direct competition of the *HOX* complexes for other transcriptional activators, but it may also involve additional mechanisms such as the recruitment of histone deacetylase to convert chromatin to an inactive state [14]. *HOX* target repression may also occur in a *PBX*/*MEIS* independent manner, by blocking the histone acetyl transferase activity of the CBP protein and, again, preventing chromatin remodelling [15].

This complex mode of transcriptional regulation may account for the equally complex and often paradoxical behaviour of *HOX* genes. One example of particular note is *HOXB4*, a gene that has recently attracted widespread attention due to its apparent ability to maintain and expand haemopoietic stem cell (HSC) populations. *HOXB4* also has numerous roles in the early embryo, some of which seem to be the direct opposite of its function in the adult.

2. HOXB4 in development

One of the most extensively studied *HOX* genes is *HOXB4*. This has a key role in determining the identity of parts of the vertebrate nervous and skeletal system during development [16–19]. *Deformed* (*Dfd*), the *Drosophila* homologue of *HOXB4*, seems to have analogous functions in the fly embryo as *Dfd* mutants lack maxillary and mandibular structures [20]. In addition to defining cell identity, *Dfd* also acts as pro-apoptotic gene during early developmental stages, maintaining the boundary between maxillary and mandibular head lobes by inducing the bordering cells to enter the apoptotic pathway through activation of the *Reaper* gene [21].

Curiously, there was until recently little evidence to suggest a comparable function for the vertebrate *HOXB4* gene (or indeed any *HOX* gene), although some cell types that would normally undergo apoptosis escape this fate in the developing brains of mice lacking a functional *Hoxa1* gene [22]. A more direct demonstration for an apoptopic role of HOXB4 comes from a recent study showing that *HOXB4* expression in the notochord cells of *Xenopus* activates cell death [23]. The notochord is an early embryonic structure consisting of a rod of cells that underlie the neural tube. Early on in development, it has a number of functions that include patterning the neural tube, but it subsequently degenerates leaving a remnant to form the nuclei pulposi that in turn give rise to

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the intervertebral discs. Some of the notochord cells undergo apoptosis and *HOXB4* is necessary for this to occur. One of the target genes for *HOXB4* in this context is *FLASH*, a component of the *FAS* mediated cell death pathway [23]. Hence, both *Dfd* and *HOXB4* activate apoptosis during development. We should perhaps be cautious in taking this parallel too far, however, as the notochord bears no embryonic relationship to the cells that separate the head lobes in *Drosophila*, and in addition, *Dfd* activates an apoptopic pathway, via modulation of the *Reaper* gene, that is distinct from that activated by *HOXB4* [21].

3. HOXB4 in adult stem cells

In addition to the numerous roles in development described above, *HOXB4* is also a key regulator of HSCs in the adult. Ectopic *HOXB4* expression is sufficient to confer the definitive adult HSC characteristics on embryonic progenitor cells [24]. Furthermore, retrovirally driven *HOXB4* expression in transduced adult HSC populations leads to a 1000-fold net increase in growth [25], without any apparent reduction in the ability of these cells to subsequently differentiate into lymphoid and myeloid lineages [24,25], and without the cells becoming transformed. The in vivo significance of this finding is reflected in the impaired haemopoiesis in mice lacking the *HOXB4* gene [26] and in the apparent ability of HOXB4 protein itself to expand HSC populations ex vivo [27].

How does *HOXB4* promote HSC proliferation? The answer presumably lies in the downstream target genes which are activated or repressed in *HOXB4* expressing cells. Several studies have indicated that the *PBX* co-factor is not required for the proliferative effects of *HOXB4* and may in fact limit *HOXB4*-induced proliferation [28,29]. Despite this, *HOXB4* expression in HSC activates the transcription of a number of genes involved in the regulation of cell division, including *c-myc* [30]. Forced expression of *c-myc* mirrors the effects of *HOXB4* expression in HSCs, suggesting that it is actually sufficient to mediate *HOXB4* action. Interestingly, *c-myc* is also strongly upregulated by *Notch*, a membrane ligand/receptor protein that promotes HSC proliferation in a similar manner to *HOXB4* [30]. In addition to *c-myc*, two components of the AP-1 transcriptional activation complex, *Jun-B* and *Fra-1*, are also directly upregulated by *HOXB4*, and their overexpression can likewise replicate the proliferative effects of *HOXB4* [31] (see Fig. 1).

4. Differential activity of HOXB4

HOXB4 is not the first example of a transcription factor that can both activate and repress apoptosis. One of the best characterised is the *Rel/NF-κB* transcription factor, which has both anti- and proapoptopic roles in T-cells (reviewed in [32]). Like *HOXB4*, *Rel/NF-κB* promotes apoptosis by activating the transcription of proapoptopic genes and can block apoptosis by the direct activation of antiapoptopic genes. Unlike *Rel/NF-κB* however, *HOXB4* does not just repress apoptosis in some circumstances, but actively promotes proliferation. What are the key differences in the cellular environments that allow *HOXB4* to function in such drastically different ways? There are probably numerous possibilities, but the two that seem most likely are summarised in Fig. 2. The first of these is a difference in the expression of *HOX* co-factors in different contexts (Fig. 2A). This certainly seems to be important in the modulation of Rel/NF-κB activity [33,34]. Furthermore, it is clear that co-factors such as *PBX* and *MEIS* significantly alter the functionality of *HOX* proteins, both directly and indirectly, primarily to activate gene transcription. Other co-factors, for example histone deacetylase, may mediate transcriptional repression. The activity of HOX proteins may then be modu-

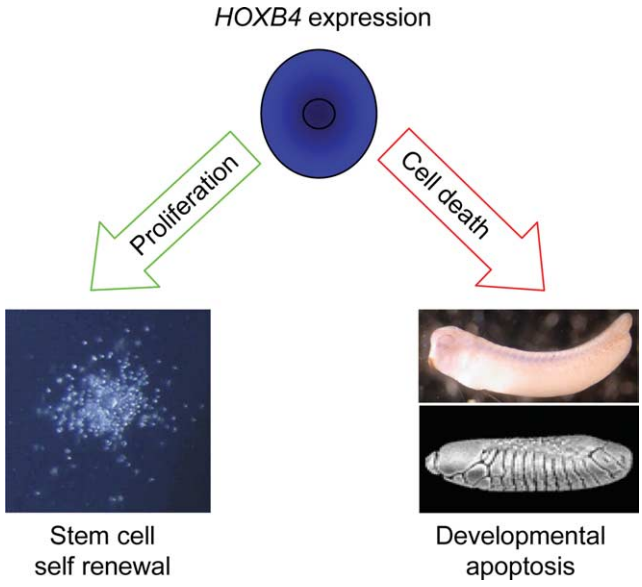

Fig. 1. *HOXB4* can activate either proliferation or apoptosis depending on the identity of the cell that expresses it. Drosophila image reproduced with the kind permission of Rudolf Turner, University of Indiana.

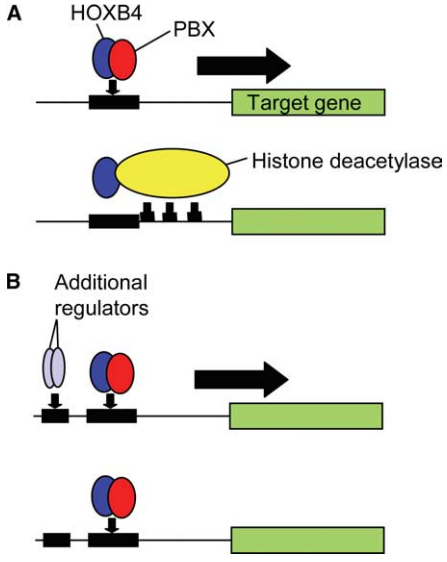

Fig. 2. Modulating *HOXB4* activity. (A) Differential availability of co-factors. HOXB4 (blue oval) may activate the transcription of a target gene when present as a complex with, for example, PBX (red oval). Alternatively, transcription could be blocked by its association with other co-factors such as histone deacetylase (yellow oval). (B) The activation of transcription may require additional factors (lilac oval) to bind to other sites in the genes enhancer, and these factors may only be available in specific cells and tissues.

lated by the presence of distinct sets of co-factors expressed in different cells. There is at present little evidence to suggest that this form of control is important in vivo, and indeed one study has shown that *HOXB4* can repress the transcription of the *Rap1* gene in cells which do express *PBX* and *MEIS* [35].

A second possibility is that, due to the inherent multi-component nature of transcriptional activation, HOX proteins will differentially activate target genes depending on the presence of other enhancer binding proteins (Fig. 2B). A possible example of this could be the regulation of *FLASH*, a pro-apoptotic gene in the notochord of *Xenopus* embryos. *HOXB4* is not only expressed in the notochord, but also in surrounding tissues of the neural tube and the somitic mesoderm [17]. Despite this, *FLASH* expression is upregulated only in notochord cells by *HOXB4* [23]. The notochord is quite distinct from its neighbouring tissues, however, and it expresses, for example, a number of notochord-specific transcription factors. It is possible then that *FLASH* transcription depends not only upon *HOXB4*, but also upon a number of other components that are specific to the notochord. Deletion and mutation studies of the *FLASH* promoter region, together with better characterisation of notochord-specific transcription factors, may help us gain a better understanding of this form of control.

Of course, both or neither of these models may explain differential *HOXB4* activity in different contexts, and to date there have been no comprehensive studies to establish which occur in vivo. What is clear though is that the possible therapeutic use of HOXB4 protein needs to be considered carefully in light of the very different responses that cells have to this multifunctional gene. Hopefully, further studies will help us to understand better these diverse, context dependent properties of *HOXB4*.

References

[1] Krumlauf, R. (1994) Hox genes in vertebrate development. Cell 78, 191–201.
[2] Carroll, S.B. (1995) Homeotic genes and the evolution of arthropods and cordates. Nature 376, 479–485.
[3] Gehring, W.J. (1988) Master Control Genes in Development and Evolution: The Homeobox Story, Yale University Press, New Haven and London.
[4] Burke, A.C. (2000) Hox genes and the global patterning of the somitic mesoderm. Curr. Top. Dev. Biol. 47, 155–181.
[5] Chang, C.P., Shen, W.F., Rozenfeld, S., Lawrence, H.J., Largman, C. and Cleary, M.L. (1995) Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins. Genes Dev. 9, 663–674.
[6] Chang, C.P., Brocchieri, L., Shen, W.F., Largman, C. and Cleary, M.L. (1996) PBX modulation of HOX homeodomain amino-terminal arms establishes different DNA-binding specificities across the HOX locus. Mol. Cell Biol. 16, 1734–1745.
[7] Chang, C.P., Jacobs, Y., Nakamura, T., Jenkins, N.A., Copeland, N.G. and Cleary, M.L. (1997) Meis proteins are major in vivo DNA binding partners for wild-type but not chimeric Pbx proteins. Mol. Cell. Biol. 17, 5679–5687.
[8] Hayashi, S. and Scott, M.P. () What determines the specificity of action of Drosophila homeodomain proteins? Cell 63, 883–894.
[9] Jacobs, Y., Schnabel, C.A. and Cleary, M.L. (1999) Trimeric association of Hox and TALE homeodomain proteins mediates Hoxb2 hindbrain enhancer activity. Mol. Cell Biol. 19, 5134–5142.
[10] Ryoo, H.D. and Mann, R.S. (1999) The control of trunk Hox specificity and activity by Extradenticle. Genes Dev. 13, 1704–1716.
[11] Vlachakis, N., Choe, S.K. and Sagerstrom, C.G. (2001) Meis3 synergizes with Pbx4 and Hoxb1b in promoting hindbrain fates in the zebrafish. Development 128, 1299–1312.
[12] White, R.A., Aspland, S.E., Brookman, J.J., Clayton, L. and Sproat, G. (2000) The design and analysis of a homeotic response element. Mech. Dev. 91, 217–226.
[13] Berkes, C.A., Bergstrom, D.A., Penn, B.H., Seaver, K.J., Knoepfler, P.S. and Tapscott, S.J. (2004) Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential. Mol. Cell 14, 465–477.
[14] Lu, Y., Goldenberg, I., Bei, L., Andrejic, J. and Eklund, E.A. (2003) HoxA10 represses gene transcription in undifferentiated myeloid cells by interaction with histone deacetylase 2. J. Biol. Chem. 278, 47792–47802.
[15] Shen, W.F., Krishnan, K., Lawrence, H.J. and Largman, C. (2001) The HOX homeodomain proteins block CBP histone acetyltransferase activity. Mol. Cell. Biol. 21, 7509–7522.
[16] Graham, A., Papalopulu, N., Lorimer, J., McVey, J.H., Tuddenham, E.G. and Krumlauf, R. (1988) Characterization of a murine homeo box gene, Hox-2.6, related to the *Drosophila* deformed gene. Genes Dev. 2, 1424–1438.
[17] Godsave, S., Dekker, E.J., Holling, T., Pannese, M., Boncinelli, E. and Durston, A. (1994) Expression patterns of Hoxb genes in the Xenopus embryo suggest roles in anteroposterior specification of the hindbrain and in dorsoventral patterning of the mesoderm. Dev. Biol. 166, 465–476.
[18] Ramirez-Solis, R., Zheng, H., Whiting, J., Krumlauf, R. and Bradley, A. (1993) Hoxb4 (Hox-2.6) mutant mice show homeotic transformation of a cervical vertebra and defects in the closure of the sternal rudiments. Cell 73, 279–294.
[19] Hooiveld, M.H., Morgan, R., in der Rieden, P., Houtzager, E., Pannese, M., Damen, K., Boncinelli, E. and Durston, A.J. (1999) Novel interactions between vertebrate Hox genes. Int. J. Dev. Biol. 43, 665–674.
[20] Merrill, V.K., Turner, F.R. and Kaufman, T.C. (1987) A genetic and developmental analysis of mutations in the deformed locus in *Drosophila melanogaster*. Dev. Biol. 122, 379–395.
[21] Lohmann, I., McGinnis, N., Bodmer, M. and McGinnis, W. (2002) The *Drosophila* Hox gene deformed sculpts head morphology via direct regulation of the apoptosis activator reaper. Cell 110, 457–466.
[22] del Toro, E.D., Borday, V., Davenne, M., Neun, R., Rijli, F.M. and Champagnat, J. (2001) Generation of a novel functional neuronal circuit in Hoxa1 mutant mice. J. Neurosci. 21, 5637–5642.
[23] Morgan, R., Nalliah, A. and Morsi El-Kadi, A. (2004) FLASH, a component of the FAS/CAPSASE8 apoptotic pathway, is directly regulated by Hoxb4 in the notochord. Dev. Biol. 265, 105–112.
[24] Kyba, M., Perlingeiro, R.C. and Daley, G.Q. (2002) HoxB4 confers definitive lymphoid–myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell 109, 29–37.
[25] Antonchuk, J., Sauvageau, G. and Humphries, R.K. (2002) HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell 109, 39–45.
[26] Brun, A.C., Bjornsson, J.M., Magnusson, M., Larsson, N., Leveen, P., Ehinger, M., Nilsson, E. and Karlsson, S. (2004) Hoxb4-deficient mice undergo normal hematopoietic development but exhibit a mild proliferation defect in hematopoietic stem cells. Blood 103, 4126–4133.
[27] Amsellem, S., Pflumio, F., Bardinet, D., Izac, B., Charneau, P., Romeo, P.H., Dubart-Kupperschmitt, A. and Fichelson, S. (2003) Ex vivo expansion of human hematopoietic stem cells by direct delivery of the HOXB4 homeoprotein. Nat. Med. 9, 1423–1427.
[28] Krosl, J., Beslu, N., Mayotte, N., Humphries, R.K. and Sauvageau, G. (2003) The competitive nature of HOXB4-transduced HSC is limited by PBX1: the generation of ultra-competitive stem cells retaining full differentiation potential. Immunity 18, 561–571.
[29] Beslu, N., Krosl, J., Laurin, M., Mayotte, N., Humphries, R.K. and Sauvageau, G. (2004) Molecular interactions involved in HOXB4-induced activation of HSC self-renewal. Blood [Epub ahead of print].

[30] Satoh, Y., Matsumura, I., Tanaka, H., Ezoe, S., Sugahara, H., Mizuki, M., Shibayama, H., Ishiko, E., Ishiko, J., Nakajima, K. and Kanakura, Y. (2004) Roles for c-Myc in self-renewal of hematopoietic stem cells. J. Biol. Chem. 279, 24986–24993.
[31] Krosl, J. and Sauvageau, G. (2000) AP-1 complex is effector of Hox-induced cellular proliferation and transformation. Oncogene 19, 5134–5141.
[32] Kucharczak, J., Simmons, M.J., Fan, Y. and Gelinas, C. (2003) To be, or not to be: NF-kappaB is the answer-role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene 22, 8961–8982.
[33] Edelstein, L.C., Lagos, L., Simmons, M., Tirumalai, H. and Gelinas, C. (2003) NF-kappa B-dependent assembly of an enhanceosome-like complex on the promoter region of apoptosis inhibitor Bfl-1/A1. Mol. Cell. Biol. 23, 2749–2761.
[34] Rocha, S., Campbell, K.J. and Perkins, N.D. (2003) p53- and Mdm2-independent repression of NF-kappa B transactivation by the ARF tumor suppressor. Mol. Cell 12, 15–25.
[35] Morsi El-Kadi, A.S., in der Reiden, P., Durston, A. and Morgan, R. (2002) The small GTPase Rap1 is an immediate downstream target for Hoxb4 transcriptional regulation. Mech. Dev. 113, 131–139.