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Molecules in focus
The HOXC6 homeodomain-containing proteins

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Abstract

The HOXC6 homeodomain-containing proteins act as transcription factors in the genetic control of multiple genes involved in development and cell differentiation. Two HOXC6 polypeptides are encoded by a single homeobox ('*HOX*') gene described as 'master gene' for the crucial role it plays in the patterning and axial morphogenesis of multiple species. Transcription of the *HOXC6* gene is initiated from two promoters and generates two proteins that share the same DNA-binding domain but harbor a distinct N-terminal region. Recent studies have demonstrated that both HOXC6 products can activate or repress transcription, depending on the cellular context. Functional in vivo specificity of HOXC6 proteins may be achieved through combinatorial interactions with other members of the HOX family as well as with co-factors whose identities are largely unknown. Disruption of this 'HOX code' may lead to pathology such as developmental defects.

Keywords: Homeodomain; Transcription; Development

1. Introduction

Molecular characterization of actors regulating the genetic programs involved in development have lead to the identification of the homeobox region defined as a 183 bp highly conserved sequence [1] that is shared by 39 members of the *HOX* gene family (see Ref. [2] for a recent review). These genes are organized in four distinct clusters (loci A, B, C and D) located on chromosome 7, 17, 12 and 2, respectively [3] (Fig. 1) and encode DNA-binding proteins acting as transcription factors [4]. Their chromosomal localization is associated with their spatio–temporal pattern of expression in the developing embryo ('colinearity') [5]. A human HOXC6 cDNA clone, previously named HOXc8.5111, has been isolated from SV-40 transformed fibroblasts [6] whereas a second HOXC6 cDNA has been recently obtained from a human breast cancer-derived cell line [7].

2. Structure

Genomic organization and expression of the *HOXC6* gene have been investigated in some species including *Xenopus* [8], mouse [9] and human [6, 7]. It appears that this member of the

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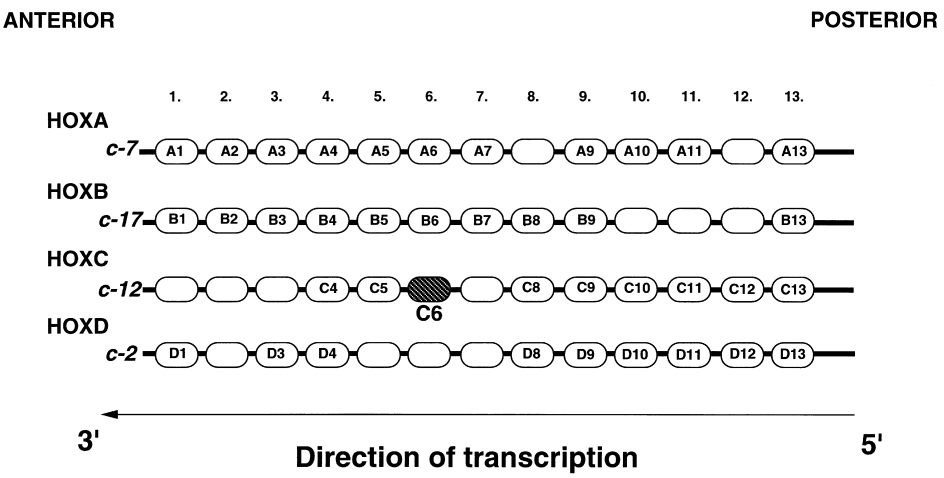

Fig. 1. Clustered organization of the 39 members of the HOX genes family. The human HOXC6 gene is located on chromosome 12 ('cluster C').

HOX family displays two promoters, named PRI and PRII and located 9 kb apart (Fig. 2). The differential use of both promoters leads to the synthesis of a 2.2 kb transcript encoding a 153 amino acids product ('short protein') and of a second 1.8 kb mRNA which codes for a 235 amino acids polypeptide ('long protein'). As illustrated in Fig. 2, both *HOXC6* gene products are translated using the same open reading frame but harbor distinct N-terminal regions because of the use of a different initial ATG. However, both products share the same 61 amino acid DNA-binding sequence ('homeodomain') as well as an identical 'pentapeptide' defined as a small sequence located upstream the homeodomain and required for the interaction of the *HOX* gene products with other proteins such as Pbx [10].

These products are highly conserved throughout evolution since both human and mouse long HOXC6 proteins are 99% similar and display an identical homeodomain sequence.

3. Biological function

Expression studies have demonstrated that the homeodomain-containing proteins are spatio–temporally regulated along the antero–posterior axis of *Xenopus* and mouse embryos [11]. Moreover, gain of function experiments cause homeotic transformations in a Hoxc6 transgenic mouse [12]. Taken together, these results strongly suggest that this homeobox gene, as well as other members of this family, play a central role in positional specification during embryogenesis of multiple species. Moreover, an additional role for these gene products in cell differentiation has been suggested by studies demonstrating *HOXC6* gene expression in a variety of adult tissues, including kidney, normal and neoplastic colon, small-cell lung cancers [13], normal mammary gland and breast carcinoma [7]. Since both HOXC6 transcripts are differentially expressed in embryonic [11] as well as in adult tissues, they may play different roles in transcriptional regulation by interacting with distinct partners. Indeed, antagonistic roles for long and short HOXC6 proteins have been proposed based on distinct phenotypes obtained in the *Xenopus* by microinjection experiments [14].

Although functional HOXC6 domains have not been formerly characterized, it is now well established that *HOXC6* gene products are transcription factors that modulate the expression of multiple target genes such as the one encoding the neural cell adhesion molecule (N-CAM) in

Xenopus [15] and other genes yet to be identified. Initial studies have demonstrated that both *Xenopus* HOXC6 products activate the transcription [15] whereas the human products harbor intrinsic repressing abilities in another cell type [7]. These observations suggest that HOXC6 transcription properties depend on the cellular context and may be mediated by DNA-protein as well as protein–protein interactions established with other homeodomain-containing products to create a 'combinatorial effect'. The existence of such effect is supported by overlapping domains of expression of these proteins along the antero-posterior axis of the embryo, thus implying that multiple products are expressed in a single cell ('HOX code') and probably compete for common HOX binding sites. Beside this combinatorial effect, homeodomain-containing proteins, including HOXC6 products, achieve their *in vivo* specificity by establishing interactions with cofactors such as Pbx proteins [10, 16]. It is likely that other transcription factors yet to be identified are involved in these processes.

4. Role in disease processes

HOX genes play a critical role in embryogenesis as demonstrated by the phenotypic abnormalities of the embryo caused by gene disruption experiments affecting one or two mouse *HOX* genes [2]. Thus, they have been considered as strong candidates for mutations leading to developmental defects. Two recent reports have indeed established a link between naturally occurring alterations of *HOXA13* and *HOXD13* coding sequences and inherited abnormality of limb development [17, 18]. The resulting phenotypes are very similar to those obtained by disruption of the corresponding genes [19] as well as by gain of function experiments [20, 21]. So far, it is not known whether disruption of the *HOXC6* gene sequence is associated with any developmental defects. It is however probable that other *HOX* genes, maybe including *HOXC6*, are altered in their regulating and/or coding sequences thus contributing to pathology by disruption of the 'HOX code' (Fig. 3). In the hypothetical model presented, a gain or loss of function of a given *HOX* gene causes an imbalance of the HOX protein levels and consequently to the dysregulation of their common target gene. Further studies are required to determine whether such a model can be applied to developmental defects yet to be characterized.

Several studies have demonstrated translocations affecting the coding or regulatory sequences of *HOX* genes in human cancer. In particular, a chromosomal rearrangement leading to the transcription of a fusion transcript has been detected in some myeloid leukaemia and involves *HOXA9* and a nucleoporin gene [22, 23]. Although so far

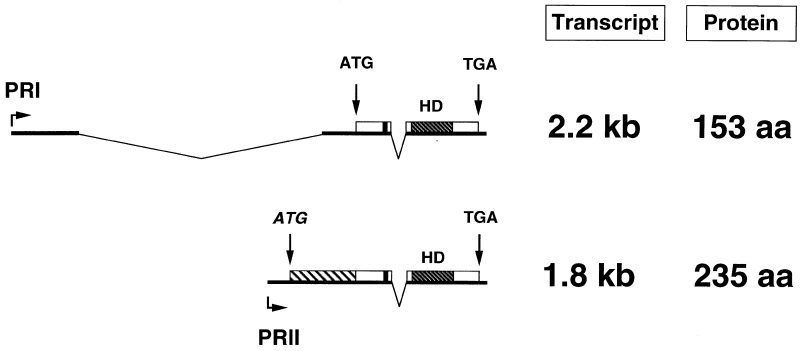

Fig. 2. Schematic representation of the exon–intron structure of both HOXC6 transcripts. Size of these transcripts as well as their corresponding gene products are mentioned. Coding sequences and untranslated regions are illustrated by rectangles and straight lines, respectively. Homeodomains (HD) and pentapeptides are represented by hatched and black rectangles, respectively. The 82 amino acid extension present at the amino terminus of the long HOXC6 product is illustrated by the stipled rectangle.

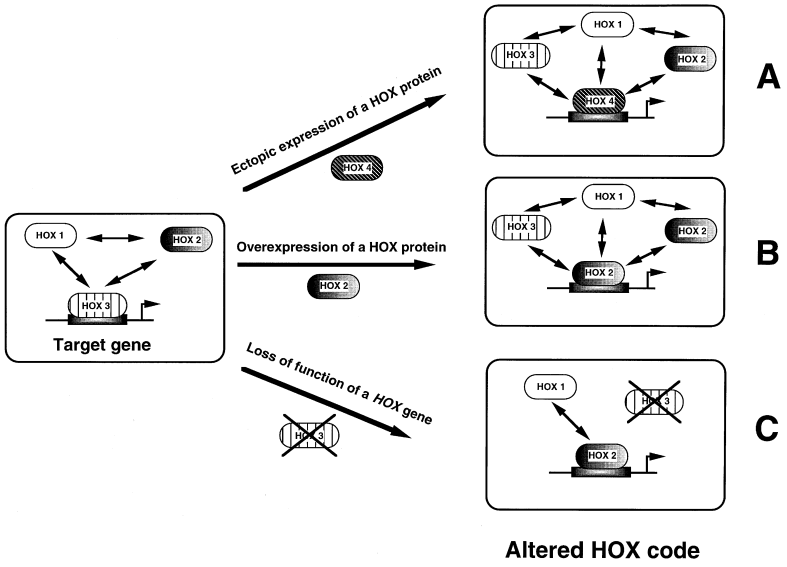

Fig. 3. Three potential alterations of the 'HOX code' leading to disease processes. A HOX-binding sequence within the promoter of a target gene is illustrated. In this cell, the 'HOX code' is defined by the expression of three homeodomain-containing proteins ('HOX 1, 2 and 3') which compete with each other for a common binding site. (A) Because of the expression of another protein ('HOX4') which is normally not present in this cell, HOX 3 is competed out of the binding site by HOX 4, an event causing the altered regulation of the target gene. (B) A local increase of the concentration of the HOX 2 protein allows this product to compete out HOX 3. (C) Loss of function of the HOX 3 gene either by deletion or mutation affecting a functional domain of the corresponding protein also leads to the binding of HOX 2 product on the site and to dysregulation of the target gene.

no alterations of *HOX* genes have been detected in solid tumors, recent reports have illustrated abnormal expression of some transcripts (see Ref. [13] for a review), including *HOXC6* [7] in tumors, thus suggesting that these transcription factors may act as oncogenes when altered in their coding sequences or ectopically expressed.

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