PERSPECTIVE

Hox is in the hair: a break in colinearity?

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During mammalian fetal development, axial structures acquire their specifications through the action of the *Hox* gene family of transcription factors (for review, see Krumlauf 1994). These are 39 such genes that are responsible for giving spatially restricted cues in a variety of embryonic derivatives, from the neural tube to the intestinal tract. In presomitic mesoderm, for example, level-specific qualitative as well as quantitative differences in HOX protein content will instruct the future sclerotomes on the morphological features that particular vertebrae should display. Therefore, it is important that the establishment of *Hox* expression domains be controlled faithfully, as slight mistakes in this process will lead to the misidentification of the corresponding structures, an effect that is often referred to as homeotic transformation. The complex coordination of this control is achieved, in part, through a unique property of this gene family; genes are organized along the chromosome in a genomic sequence that reflects their time and place of activation during development. In mammals, there are four *Hox* clusters (*A* to *D*) and within each cluster, *Hox* genes located at the 3' end are activated first and in anterior embryonic domains, whereas genes located at the 5' end are transcribed subsequently and in more caudal areas. The spatial aspect of this intriguing correspondence, or "colinearity," was described originally by Lewis (1997) in *Drosophila*, and has since been observed in all animals exhibiting an anterior-to-posterior axial polarity. Interestingly, no clear exception to this rule has been reported to date. In this issue, however, Godwin and Capecchi present some surprising results from an elegant study of murine *Hoxc13*, a very posterior gene member of the *HoxC* complex. Unexpectedly, *Hoxc13* is expressed in hair follicles throughout the body as well as in vibrissae and in the tongue, that is, at locations much too anterior for the "genomic position" of this gene. Does *Hoxc13* violate the code of colinearity?

Hox genes members of the most posterior groups of paralogy, from group 9 to 13, are all related to the *Drosophila* gene *Abdominal-B* (*AbdB*; Izpisua-Belmonte et al. 1991). Expression studies as well as gene knock outs have revealed that *Hoxd13* and *Hoxa13* are required for the development of the most posterior structures in the trunk, such as the sacrocaudal vertebral transition, the anal sphincter, the external genitalia, as well as the uro-

genital system and associated glands (Dollé et al. 1993; Davis and Capecchi 1996; Kondo et al. 1996, 1997; Podlasek et al. 1996; Warot et al. 1997). The expression of the two other members of this paralogy group, *Hoxb13* (Zeltser et al. 1996) and *Hoxc13* (Peterson et al. 1994), suggested that a similar posterior restriction would be observed in the phenotypes of mice carrying null alleles of these genes. The generation of mice lacking *Hoxc13* function confirmed that alterations of the vertebral column were restricted to the tail, where homeotic transformations of caudal vertebrae toward a more anterior morphology were observed (Godwin and Capecchi 1997). Surprisingly however, homozygous animals lacked vibrissae at birth and subsequently showed a strong defect in brittle hair leading to alopecia, that is, the absence of emerging hairs, although hair follicles seemed to form normally. Because the inactivation strategy involved the insertion of *lacZ* reporter sequences in-frame with the *Hoxc13* coding sequences, a detailed expression study allowed a perfect correlation to be established between this unexpected phenotype and *Hoxc13* transcription specificity during fetal skin development.

Hox is in the hair

The morphogenesis of vertebrate skin involves interactions between the dermis and the epidermis, a mesenchyme and an epithelium, respectively. Various region-specific appendages are produced by this organ such as vibrissae and pelage (body coat) hairs. Recombination experiments by Dhouailly (1977) have shown that this regional specificity is linked mainly to the origin of the dermis. The morphogenetic steps in the development of vibrissae and pelage hair follicles are similar to each other and have been classified by Hardy (1968; see also Kantzler et al. 1994). Differentiation of the facial integument begins at day 11.5 (E11.5), whereas body skin differentiation does not occur before E14.5. Initially, a condensation of the dermis appears together with a thickening of the epithelial layer. Epidermal cells then proliferate to produce hair pegs and keratinocytes will differentiate to form the hair follicles followed by the emergence of hair shafts at E18. Although the expression of *Hoxc13* was not detected in dermal papillae, the use of various markers such as a type II epidermal keratin (K2.6), which together with its partner K1.16 characterizes proliferative keratinocytes, allowed the detection of β -galactosidase (β -gal) activity in all hair follicles (God-

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win and Capecchi 1997), in those cells of the companion layer expressing the K2.6 keratin (Rothnagel and Roop 1995). Interestingly, this single cell layer was still present in *Hoxc13* homozygous mutant animals, indicating that this function was not required for the production or maintenance of these cells. *Hoxc13* transcription was then detected in rapidly dividing keratinocytes in the hair bulb as well as in the hair shaft, concomitant with the expression of high sulfur proteins (Lynch et al. 1986).

β -Gal activity was also scored in the filiform papillae of the tongue, structures that are specific for the dorsal surface of the tongue and thought to resemble hair follicles due to the presence of papillae, a cross-reactivity with an antibody against differentiating hairs (Dhouailly et al. 1989), and to the expression of several genes such as those for hair keratin intermediate filaments and trichohyalin. In these filiform papillae, *Hoxc13* was expressed as early as E17 in a region closely corresponding to that of *mHa3* hair keratin expression (Winter et al. 1994). Such a widespread expression of *Hoxc13* in these cutaneous appendages correlated nicely with a series of striking phenotypes affecting all of these different structures such as a lack of protruding vibrissae and abnormal nails. Surviving mice were hairless, and essentially all hair types described so far (Sundberg and Hogan 1994) were affected by this mutation. Although pelage hairs formed, they were fractured at the skin surface, so that some rare hairs could be detected in regions protected from rubbing. Likewise, filiform papillae of the tongue were broken. Even though no particular type of keratin was shown to be affected in mutant hair follicles, such a phenotype strongly suggested a function for *Hoxc13* in controlling the production of structural proteins necessary for the differentiation of skin appendages. The wide distribution of these phenotypic alterations genetically demonstrates the ontogenetic relationships between the different skin appendages.

A violation of colinearity?

Expression of *Hox* genes in developing skin has been reported previously (Chuong et al. 1990; Bieberich et al. 1991; Kantzler et al. 1994). However, an anterior-posterior restriction in the extent of expression along the rostrocaudal axis was observed. In the case of the closely related *Hoxd13* gene, expression was reported in epithelial-derived portions of hair follicles but only in the most caudal part of the skin. In this case, the expression boundary was indistinguishable from those of *Hoxd9* and *Hoxd11*, which were also expressed in these integuments (Kantzler et al. 1994). What makes the Godwin and Capecchi report of particular interest, in addition to the functional aspect discussed above, is that *all* hair follicles are involved, regardless of their positions along the body axis. Godwin and Capecchi emphasize that such an observation apparently is at odds with the rule of spatial colinearity, which would have predicted an expression of *Hoxc13* in caudal hair follicles only (see Fig. 1). However, a slightly different view of these results suggests that if a violation of colinearity is observed, it

Figure 1. Schematic representation of the expression patterns of the two paralogous genes, *Hoxd13* and *Hoxc13*, at different developmental stages. In the *middle*, the posterior halves of the *HoxD* (top) and *HoxC* (bottom) complexes are shown with the five genes related to the *Drosophila AbdB* gene (from group 9 to 13). The expression profiles of these two genes, either at E9 (left) or at a later developmental stage (right), are depicted. (Left) Both genes show an early activation (as represented in black) in the region of the tail bud, thus respecting colinearity. On the right, a clear difference appears in hair follicles. Although *Hoxd13* expression is restricted to the caudal part (black dots), *Hoxc13* is expressed there up to anterior regions, as well as in the vibrissae. Other areas of expression are not shown. The two diagrams on the right merely illustrate a general developmental stage (see the text, and Godwin and Capecchi 1997, for precise details). Similarly, the labeled hair follicles do not reflect a genuine pattern but indicate the absence of posterior restriction in *Hoxc13*.

may not be so serious and, most importantly, does not imply a revision of this concept. In particular, the original colinear mechanism, that is, the process by which *Hox* genes are sequentially activated temporally and spatially as gastrulation proceeds, is clearly respected. During early development, *Hoxc13* expression was as anticipated, appearing rather late in the growing tail bud, that is, with a strict posterior restriction (Fig. 1; Peterson et al. 1994; Godwin and Capecchi 1997). In this respect, therefore, colinearity as defined originally to apply to vertebrates (Gaunt et al. 1988; Duboule and Dollé 1989; Graham et al. 1989) is not transgressed by *Hoxc13*, and the subsequent widespread expression of this gene in hair follicles is not connected to its early colinear regulation.

Such an unexpected expression pattern could be best explained by the functional recruitment of this gene, in the course of evolution, for achieving some additional function in hair follicles such as controlling the production of structural proteins, as proposed by Godwin and

Capecchi (1997). The close linkage of the *keratin-2* complex to the *HoxC* locus on mouse chromosome 15 (Hart et al. 1992) may be indicative of how some *Hox* genes became involved in hair development, through the potential sharing of a hair follicle-specific global regulatory control. The phenotype indicates that proteins involved in the solidity of the hairs could be under the control of *Hoxc13*, such as some high sulfur proteins associated with keratin intermediate filaments (Lynch et al. 1986). *Hox* cream may cure baldness.

An alternative possibility is that *Hox* genes are expressed in hair follicles to establish a polarity in these structures. In this view, hair follicles would be considered as axial structures and would then require the *Hox* information to differentiate proximal from distal aspects. In this case, a break in colinearity would not be unexpected. Such a situation has already been observed with *Hoxd13* and *Hoxa13*, two genes strongly expressed in forelimbs, that is, at more anterior positions than their transcript domains in the trunk (Dollé et al. 1993; Haack and Gruss 1993). In fact, it is shown here that *Hoxc13* is also expressed in forelimbs; hence the proposed break in colinearity may not be restricted to hair follicles. In support of this view, *Hoxc13* seems to be required for one of the last steps in hair differentiation (Godwin and Capecchi 1997), much in the same way that *Hoxd13* and *Hoxa13* are involved in the last steps of limb pattern formation (Fromental-Ramain et al. 1996). However, it is unlikely that *Hox* gene expression in the course of hair development follows the same strategy as for either the trunk or the appendicular axes. In the case of *Hoxc8*, another member of the *HoxC* complex, although expression in the epidermal component of the skin was also scored, it was found only at restricted body levels and two glabrous zones were observed in the lumbar region of mice homozygous for a null allele of this gene (Kanzler et al. 1994). In addition, *Hoxc8* was reported to be transcribed in the dermal papillae, in marked contrast to *Hoxc13*. This finding reinforces the suggestion that the function of *Hoxc13* during hair development may not reflect a general feature of most *HoxC* complex genes. Furthermore, during skin development, posterior *Hoxd* genes are expressed in caudal areas exclusively, indicating that whatever their function is in the morphogenesis of integuments, this function is not required throughout the body, unlike what is now reported for *Hoxc13*. Hoxwise, hair follicles do not seem to share the properties of genuine appendicular structures such as the limb and genital buds.

The Hox veto

It is likely that a closer examination of *Hox* gene expression and function will reveal many target tissues and organs in adults, in which particular HOX proteins will be necessary, regardless of their original embryonic, colinear distribution. This is to be expected when considering the high degree of “recycling” of control genes, as judged by their commonly observed multiple sites of expression and pleiotropic effects (for review, see Gerhart

and Kirschner 1997). One might even wonder why, during the course of evolution, such a reservoir of *Hox* transcription factors, with a rather large spectrum of DNA binding specificities, has not been used more frequently as a source of material for early development. This question is particularly valid for early (pregastrulation) embryonic development. Indeed, *Hox* function seems not to be required until about day 7 postfertilization, the time at which the anterior *Hox* genes first become activated. It is puzzling that the many *Hox* genes that are involved in processes unrelated to their original axial-specification function (e.g., in blood cells or during spermatogenesis) achieve these additional functions only *after* their most ancestral task is over (i.e., after gastrulation), whereas none of these genes was ever reported to affect embryonic development *before* their colinear activation. It is as if the functional recruitment of *Hox* genes in vertebrates had been made impossible for a function occurring before axial specification.

One possible reason for this strange observation may rely directly upon the mechanistic basis of colinearity. It has been proposed that vertebrate *Hox* complexes, early in development, are in a closed configuration and that the colinear activation of these genes follows their progressive accessibility through a linear “opening” of the complexes (van der Hoeven et al. 1996). In such a view, a functional recruitment of a *Hox* gene for an earlier function, for instance, mesoderm induction, would necessitate the premature opening of the complex during development; hence, the loss of this critical property. This situation would be naturally detrimental to the embryo as posterior HOX proteins would antagonize the functions of the anterior genes (Duboule and Morata 1994). Therefore, the mechanism behind colinearity may represent a strong functional veto to gene co-option. In contrast, diptera probably activate their homeotic genes from fully accessible complexes, as a result of the action of upstream genes already regionally expressed in the embryo (Lawrence 1992). Consequently, *Drosophila* genes required for early development are allowed to be interspersed among homeotics, such as *fushi tarazu* and *bicoid* in the *Drosophila* Antennapedia complex (e.g., Kaufman et al. 1990). In this context, an authentic violation of colinearity in vertebrates would require the expression of any *Hox* gene either before its normal time of activation or at an unusually anterior position at gastrulation. Until such expression can be documented, the rule will stand.

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