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Hox in the Niche Controls Hairy-geneity

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Patterns of mammalian hair growth vary in different regions of the body, but the mechanisms controlling this heterogeneity are unclear. In this issue of *Cell Stem Cell*, Yu et al. (2018) show that *Hoxc* gene expression in hair follicle mesenchyme varies along the anterior-posterior body axis and contributes to regional differences in hair growth.

Hair follicles undergo cycles of growth, regression, rest, and regrowth that depend on stem cells in the epithelial compartment of the hair follicle and are regulated in part by signals from the mesenchymal niche, known as the dermal papilla. Hair length and thickness correlate with duration of the hair follicle growth phase and the proliferative activity of epithelial progenitor cells. Patterns of mammalian hair growth show substantial variations between different body regions. Classical skin recombination experiments, and the ability of transplanted hair follicle dermal papillae to determine hair follicle phenotype, indicate that the dermis is the source of positional information in the skin (Hardy, 1992; Jahoda, 1992). However, the underlying mechanisms are poorly understood. In this issue of *Cell Stem Cell*, Ting Chen and colleagues use genetic studies in mice to show that expression of homeobox-containing (Hox) transcription factors in the dermal papilla controls regional variation in hair follicle growth (Yu et al., 2018).

Hox genes were first identified for their key roles in establishing the anterior-posterior axis of the *Drosophila* embryo. Vertebrate *Hox* genes are contained within several genomic clusters. Within each cluster, the 3′ most genes are expressed in more anterior body regions, and the 5′ genes more posteriorly, a phenomenon termed spatial collinearity (Figures 1A and 1B). Mutation of specific *Hox* genes in mice causes homeotic transformations of vertebrae, indicating conserved functions of these factors in anterior-posterior axis development (Wellik, 2009).

Possible roles for *Hox* genes in patterning vertebrate skin were first suggested by the findings that two chick *Hox* genes, and the mouse *Hoxc8* (*Hox 3.1*) gene, are expressed in feather and hair follicle dermal papillae, respectively, in a graded fashion along the anterior-posterior body axis (Bieberich et al., 1991; Chuong et al., 1990). However, data regarding the functional implications of these observations have been lacking until now.

In the current paper, the authors investigate the molecular mechanisms underlying regional differences in hair growth between adult mouse dorsal skin, which displays periodic cycles of growth, and ear skin, where the follicles regress after postnatal day 11 and remain dormant. In mice carrying the dominant *Koala* (*Koa*) mutation, caused by an inversion on chromosome 15, ear hair follicles remain in the growth phase for longer than usual, and, unlike normal ear hair follicles, subsequently undergo multiple cycles of regenerative growth. Among genes lying within 1 MB of the distal and proximal breakpoints, *Hoxc4*, *5*, *6*, *8*, and *9* are upregulated in *Koa* mutant ear dermal cells. The authors show that *Hoxc4* and *Hoxc6* as well as *Hoxc8* are expressed in the dermal papilla in wild-type dorsal and *Koa* mutant ear hair follicles, but not in wild-type ear follicles. Interestingly, and in line with earlier observations (Bieberich et al., 1991), *Hoxc* cluster genes show spatial collinearity in the skin, with *Hoxc4–10* being expressed in dorsal dermis, and *Hoxc10–13* in tail skin (Figure 1C). CRISPR/Cas9-mediated deletion of the *Hoxc* cluster on the *Koa* mutant chromosome prevents abnormal ear follicle growth, while a deletion that removes genomic sequences between *Hoxc13* and *Hoxc4*, but creates a *de novo Hoxc13/Hoxc4* gene fusion, fails to do so, demonstrating that the long ear hair phenotype does not result from overexpression of intergenic elements. Deletion of *Hoxc8*, the most highly expressed *Hox* gene in dorsal skin dermal papillae, is accompanied by upregulated expression of other *Hoxc* family members, but nevertheless partially rescues the *Koa* phenotype. Conversely, forced expression of *Hoxc8* in wild-type ear dermal papillae partially replicates the *Koa* phenotype. These data demonstrate that ectopic expression of *Hoxc* family members in the hair follicle dermal papilla drives abnormal ear hair growth.

The authors further show that expression of *Hoxc* genes is normally repressed in ear skin by polycomb-dependent mechanisms, and that mice lacking the polycomb complex protein BMI1 have increased expression of *Hoxc* genes and longer-than-normal ear hair. ChIP-seq for CCCTC-binding factor (CTCF), which marks the boundaries of topologically associated domains (TADs), and circularized chromatin conformation capture with *Hoxc4* as bait, show that the *Koa* inversion disrupts TADs in dermal cells and places the *Hoxc* cluster in an active regulatory landscape. RNA-seq revealed downregulation of the Wnt/β-catenin pathway activator *Rspo2*, and upregulation of the Wnt inhibitor genes *Wif1* and *Sfrp2*, in wild-type ear compared with dorsal skin. Wnt/β-catenin signaling is required for hair follicle regenerative growth (Choi et al., 2013); thus Wnt activation downstream of *Hoxc* gene expression might mediate the *Koa* phenotype. In line with this, the authors detected Wnt activity in resting hair follicles of dorsal and *Koa* mutant ear skin, but not in wild-type ear skin, and *Sfrp2* is downregulated and *Rspo2* upregulated in *Koa* mutant compared with wild-type ear skin.

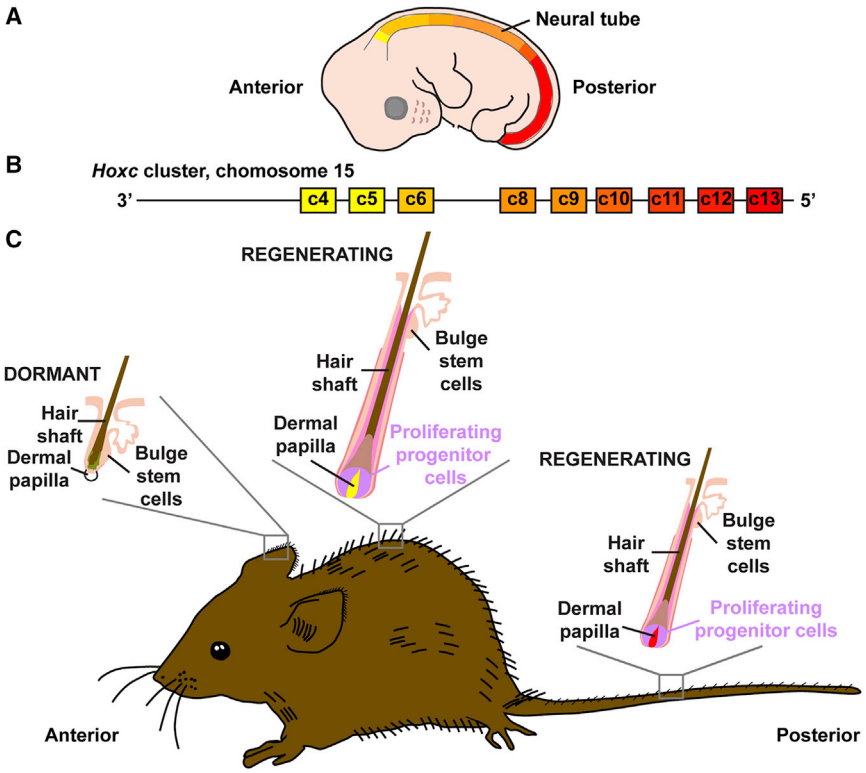

Figure 1. Schematic of *Hoxc* Gene Expression in Embryonic Mouse Neural Tube and Adult Hair Follicle Dermal Papillae

(A) Diagram of a mouse embryo at embryonic day 12.5 showing the pattern of *Hoxc* gene expression in the neural tube.

(B) Schematic of the *Hoxc* gene cluster.

(C) Hair follicles in the indicated regions of adult mouse skin: hair follicles in ear skin are in the resting phase and lack *Hoxc* expression in the dermal papilla; dorsal and tail skin hair follicles are regenerative, contain proliferating progenitor cells (violet), and express *Hoxc4–10* and *Hoxc10–13* (respectively) in their dermal papillae. Colors in the embryonic neural tube and adult dermal papillae correspond to expression of the genes indicated in the schematic of the *Hoxc* gene cluster.

The precise mechanisms by which *Hoxc* genes control Wnt pathway activity, and whether this is the only pathway affected by *Hoxc* expression, remain to be determined. For instance, mice lacking *Sfrp2* have been generated but they are not reported to display hair growth phenotypes (Satoh et al., 2006), and ear skin expresses high levels of other Wnt inhibitors and provides an environment rich in Bone Morphogenetic Factors that can inhibit hair growth (Wang et al., 2017). Interestingly, genome-wide association studies in dogs have shown that mutation of *RSPO2* associates with the presence of a moustache and eyebrows (Cadieu et al., 2009). Further delineation of the mechanisms by which *Hoxc* genes exert their effects may thus provide insight into regional hair growth phenotypes in other mammals; colinear expression of *Hox* genes in feather buds (Chuong et al., 1990) suggests that such mechanisms also apply to other vertebrate ectodermal appendages.

This study raises additional interesting questions. For instance, while tail hair follicles are regenerative, they produce smaller and finer hairs than those in dorsal skin. Future studies could investigate whether this is related to their expression of *Hoxc10–13* rather than *Hoxc4–10*. This observation, together with the fact that human scalp hair is longer and thicker than that in other body regions, runs counter to a simple model in which regenerative capacity increases along the anterior-posterior body axis, and it suggests the existence of additional layers of regulation.

In summary, the authors' fascinating findings reveal that collinear *Hox* gene expression is not only important for setting up the body plan during embryogenesis, but it is also re-utilized in the regional control of adult stem cell activity in the skin. These results provide a paradigm that may be applicable to more broadly understanding persistent regional variations in adult skin and other regenerative tissues in a wide range of vertebrate species.

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