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Splitting Hairs: Dissecting Roles of Signaling Systems in Epidermal Development

Minireview

Anthony E. Oro and Matthew P. Scott
Departments of Developmental Biology
and Genetics
Howard Hughes Medical Institute
Stanford University School of Medicine
Stanford, California 94305

Hairs in mammals and feathers in birds are two of the smallest and most fascinating organs in the body. These keratinized skin appendages serve the purposes of thermoregulation, sensation, and social interaction, and in the case of feathers, flight. Early stages of feather and hair development have both common and distinct features, so comparative studies provide useful ideas about how both structures form (thoroughly reviewed in Chuong, 1998). The variation of these organs in nature is impressive. Otter skin has 125,000 hairs per square centimeter to provide insulation and a waterproof exterior, whereas human hair is relatively sparse and mouse foot pads are devoid of hair. Peacock feathers, with their extraordinary colors and patterns, make up about 17% of the birds' body weight. How is all this controlled?

The first recognizable harbinger of the site of the future hair or feather is the placode. The placode consists of a clump of mesoderm cells underneath a small symmetric invagination of epidermis. Hair and feather development can be thought of as a two-step process. First, the number and spacing of the placodes are determined. Second, cells in the placode differentiate into several types that grow into all the structures of the mature hair or feather. The formation of thousands of placodes over the course of a 2- to 3-day period occurs in a highly reproducible order in each organism, sweeping across regions of the epidermis in spreading waves. The epidermal cells of the placode that are in contact with the underlying mesoderm continue to divide. Epidermal cells that move away from the dividing cells begin differentiating into one of the distinctive layers of the hair or into the myriad patterns and designs of the mature feather. This review focuses on very recent advances in understanding hair follicle spacing and growth, including exciting information about the effects of β-catenin, reported in this issue of *Cell* (Gat et al., 1998).

Counting and Spacing the Placodes—the Competition Model

Our understanding of the control of placode density and placement benefits from over 50 years of embryologic experimentation (reviewed in Hardy, 1992). Experiments with chick and mouse embryo skin, in which epidermis and mesoderm were recombined and transplanted, demonstrated that the site on the body from which the mesoderm originates plays a crucial early role in the spacing of hairs or feathers. These and many subsequent experiments suggested that the mesoderm induces the epidermis to produce an evolutionarily conserved signal that in turn stimulates the condensation of mesoderm cells to form the placode. Following placode initiation, one or more species-specific additional epithelial and mesodermal signals were postulated to organize the particular skin structure. A better understanding of the molecular basis of these observations has emerged from recent studies. The usual suspects for developmental biologists, the fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), Sonic hedgehog (Shh), and the Notch signaling pathway, collaborate in creating the hair. FGFs, BMPs, and Shh are secreted proteins whose binding to receptors on susceptible cells triggers signal transduction changes and transcription of target genes. Notch receptor, in contrast, is activated by membrane-bound ligands such as Delta-1 on the sending cell, resulting in transcriptional changes and induction of Notch target genes.

Feather placode formation appears to be controlled by a competition between FGF4 and BMP2 or BMP4 (Figure 1A). Transcription of all three of these genes is induced in the epithelium by the underlying mesoderm early in hair and feather placode formation. Retroviruses expressing FGF in unpatterned epithelium induce ectopic feather buds, whereas viruses driving BMP2 or BMP4 production dramatically inhibit placode formation (Jung et al., 1998; Noramly and Morgan, 1998). The role of FGF was further shown using *scaleless* mutants, featherless chickens that have a defect in the epidermis. FGF-coated beads restore wild-type feather density, indicating that FGF2 can stimulate feather formation (Song et al., 1996). In contrast, increasing doses of BMP2 or BMP4 applied on beads correlated with increasing distances between placodes (Jung et al., 1998). These experiments suggest that placodes induced by activators like FGF are surrounded by cells prevented from adopting a placode fate by the overriding activity of inhibitors such as BMP2 and BMP4.

The interpretation of bead implant and retroviral gain-of-function experiments is tricky, because the overproduced protein may have activities that differ from its roles in normal development. Loss-of-function experiments have a different problem: embryos carrying homozygous null mutations in many key genes, including those just mentioned, die at stages that preclude analysis in the skin. Tissue-specific loss-of-function experiments are needed to complement the overexpression experiments.

In feather development, the Notch pathway appears to refine the early placode pattern set up by the initial FGF/BMP competition. Delta-1, a ligand for the Notch receptor, is produced in the mesodermal condensation early in placode formation. *Delta-1* misexpression induces *Notch-1* transcription in the epidermis and has the interesting effect of promoting placode formation while inhibiting surrounding cells from adopting a similar fate (Crowe et al., 1998). In skin of the chick mutant *scaleless*, *Delta-1* is expressed uniformly throughout the dermis, but *Delta-1* can be returned to its normal pattern by the addition of an FGF source (Viallet et al., 1998). FGF may normally control where *Delta-1* is expressed relative to the new placode. Delta-1 signaling in turn contributes to placode induction and creates a boundary for the already formed mesodermal condensation, preventing recruitment of other mesodermal cells into the placode. How Delta-1 and other members of the

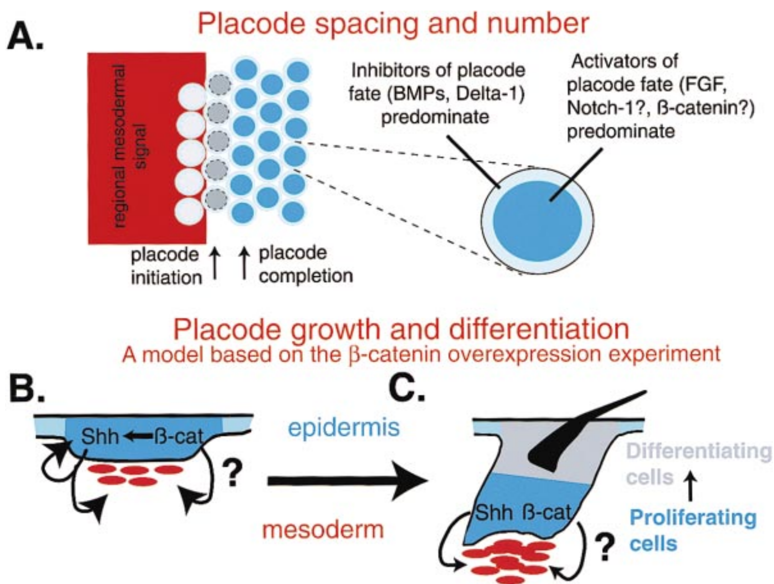

Figure 1. Model of Placode Pattern Formation and Growth Based on Mutant β-Catenin Overexpression Study

(A) Placode patterning, as seen in chick epithelium, is dictated by an initial mesenchymal signal (red) that simultaneously induces the production of both activators and inhibitors in the overlying epithelium. Placodes are formed where the activator (blue) overcomes the inhibitor (aqua). Waves of placodes are formed as the mesenchymal signal moves through the unpatterned skin (after Noramly and Morgan, 1998 and Jung et al., 1998).

(B) After the placode is formed, β-catenin induces Shh expression. Shh causes proliferation by acting in epidermal cells in which it is expressed as well as the adjacent mesoderm. β-catenin likely has many additional effects that are independent of the Shh pathway.

(C) Continual proliferation in cells expressing Shh requires additional signals, possibly from the underlying mesoderm (red) cells. Hair follicle epithelial cells that move away from the underlying mesoderm (gray) stop proliferating and begin to differentiate into the many layers of hair.

Notch pathway interact with BMPs and FGFs is an open question.

In a competition model, the observed wave of developing placodes could derive from a spreading mesodermal signal that incites the FGF-4/BMP competition as placode formation sweeps along (Figure 1A). Placode spacing would be influenced by the degree to which activators such as FGF precede (or exceed) blockers such as BMP and are then refined by the Notch pathway. The molecular basis of the migrating mesodermal signal is unknown, and no mutants have been identified where propagation of the spreading wave of placodes fails.

Epithelial Outgrowth—Tumors or Hair Follicles

The second stage of hair or feather development begins with the appearance of differentiating epidermal cells in the placode. The mesoderm cells remain as a lump at the base of the forming hair, with their differentiation revealed by changes in gene expression. The epithelial cells in contact with the mesoderm continue to divide as they are carried deeper into the skin by the invagination of the developing follicle. The epidermal cells that rise away from the mesoderm toward the surface differentiate into at least five distinct types, based upon histology and gene expression markers. In the center of the follicle, dead cells filled with keratin form the primordial hair, surrounded by two sheaths of cells, the inner and outer root sheaths. The follicle becomes densely enveloped in nerve endings, induces smooth muscles to arise from adjacent mesoderm and attach to the mid portion of the follicle, and the epidermal cells near the site of emergence of the hair at the surface become oil or sweat glands. Melanocytes migrating from the neural crest invade the epidermis to provide pigmentation to the hair. As though this is not enough, the hair forms repeatedly from the same follicle, and the follicle may harbor stem cells for the whole skin. Let no one claim that a hair is a simple model system!

The growth of the follicle is therefore largely a proliferation of epidermal cells. Although little is known about the regulation of this growth, recent evidence suggests that *Shh* plays a major role. *Shh* is expressed in epidermal cells as they invaginate into the dermis (Figure 1B). FGFs can induce *Shh* expression while BMPs suppress it, consistent with a role for Shh in responding to FGFs and BMPs by participating in placode growth in the chick epithelium (Jung et al., 1998; Noramly and Morgan, 1998). Shh can induce BMPs in chick skin, perhaps thereby limiting further placode formation.

A key antagonist of the Shh signal is the Patched (Ptc) protein, a transmembrane protein capable of binding and sequestering Hedgehog proteins (reviewed in Hammerschmidt et al., 1997). In many circumstances Shh is known to stimulate growth, while Ptc prevents it. The skin is no exception; mutations in Ptc are associated with one type of skin cancer, basal cell carcinoma. Humans and mice overexpressing Shh in their skin have similar tumors. How the growth of the tumors relates to growth of follicles is incompletely understood, but the tumors often seem to emerge from follicles, so the two types of growth may be related.

Is *Shh* controlling only growth, or does it also have effects on placode spacing or epidermal cell differentiation? Evidence exists for *Shh* involvement in both growth and placode spacing in feather formation. As in mice, *Shh* overexpression in chick skin induces tumors resembling basal cell carcinoma (Chuong, 1998; Morgan et al., 1998). A role for Shh in placode spacing is suggested by the early *Shh* expression in chick epithelium. Experimental support for the importance of the early expression comes from overexpressing *Shh* early, which causes many extra placodes to form. However, homozygous mouse *Shh* mutants have normally spaced hair follicle placodes, so *Shh* is unlikely to have an essential role in placode spacing in mouse embryos (Chiang et al., 1998; St. Jacques et al., 1998). This could be a case where hair and feathers differ, or the contradiction may be due to comparing gain-of-function with loss-of-function experiments.

Shh appears to act on both the epithelium and the dermis to exert its proliferative effects. Shh induces *ptc* transcription in many tissues, which allows *ptc* transcription to be used as an indicator of the reception of Shh. In the early hair follicle, *ptc* is transcribed in both the mesoderm and epidermis, suggesting that Shh is received by both cell types (Figure 1C). The Smoothened (Smo) transmembrane protein is required for activating transcription of Hedgehog protein target genes. Overexpression of a constitutively active Smo in the epidermis results in tumors similar to those caused by *Shh* overexpression (Xie et al., 1998). Smo affects only cells in which it is produced, in contrast to Shh, which signals to other cells, so activation of Shh targets in the epidermis, not the mesoderm, is required for growth.

There is an important twist: the tumors caused by *Shh* overexpression in skin eventually stop growing and differentiate into hair follicle structures (Oro et al., 1997). Stable proliferation, but not the initial growth of the follicle, may require signals from the underlying mesoderm. This ectoderm–mesoderm feedback loop could be analogous to one in the developing limb. Shh in the posterior limb bud mesoderm maintains FGF4 expression in the apical ectodermal ridge and vice versa; this positive feedback loop maintains limb bud outgrowth. FGF5 is normally transcribed in epidermal cells adjacent to *Shh*-expressing cells. The persistent growth of the hair shaft in mice lacking FGF5 function (Hebert et al., 1994) suggests that FGF5 blocks Shh function, in contrast to the Shh-FGF4 positive feedback in the limb. FGF5 could interfere with another FGF that reinforces Shh expression.

β-Catenin Effects: Wnt Involvement in Placode Positioning and Growth?

What other pathways affect growth and differentiation of the placode? In this issue of *Cell*, Gat et al. (1998) describe the effect on hair morphogenesis of another familiar protein, β-catenin, a protein implicated in Wnt signaling and in maintenance of cell junctions. Wnt proteins are secreted signaling molecules that control cell fates and growth in many developing tissues. β-catenin transduces Wnt signals by acting as a transcription cofactor in association with the T cell factor (TCF)/lymphoid enhancer factor-1 (Lef-1) family of transcription factors (reviewed in Willert and Nusse, 1998). β-catenin also maintains the actin cytoskeleton via its associations with cadherins. Free cytosolic β-catenin rapidly undergoes ubiquitin-dependent proteolysis mediated by the product of the tumor suppressor gene, adenomatous polyposis coli (APC), a central player in colon cancer. β-catenin protein is stabilized by a cascade of events triggered by the binding of a Wnt signal to a plasma membrane receptor. The stabilization allows β-catenin to associate with TCF/Lef-1 and induce transcription.

Gat et al. (1998) used an epidermis-specific promoter to overproduce a stable form of β-catenin in developing skin. They found a number of exciting results. The first is that remarkably complete hair development was induced in areas of epithelium between existing hair follicles, though with incorrectly angled hair, giving a higher than usual hair density. The extra follicles were well formed, containing clumps of mesoderm seen in normal hair follicles and well-differentiated hair shafts. Mutant β-catenin therefore affected the mechanism used to determine spacing and number of hairs. Second, the induction of two types of well-differentiated hair follicle tumors, classified as trichofolliculomas and pilomatricomas, occurs at a high rate. This surprising result suggests β-catenin has the novel ability to affect pattern, like FGFs and BMPs, and growth, like Shh.

β-catenin has been linked to another type of cancer: familial and spontaneous colon carcinoma. Colon tumors often have mutations in *APC* that allow the stabilization of β-catenin. The stable β-catenin presumably leads to transcription of target genes that contain TCF (hTCF-4) response elements. The link between levels of β-catenin and cancer is strengthened by the discovery that several colon tumor cell lines have wild-type *APC* genes. The cell lines carry a mutation in β-catenin that prevents its degradation, so the outcome may be the same: too much transcription of β-catenin target genes (Willert and Nusse, 1998).

The hair follicle tumors caused by overexpression of a stable form of β-catenin, and the Shh control of growth, bring to mind the Wnt-Hedgehog mutual activation that occurs in fly epidermis. Indeed, in the β-catenin-induced tumors *Shh* transcription is elevated. If *Shh* were the only target of β-catenin, then induction of either *Shh* or β-catenin should have the same outcome. They do not. The basal cell carcinomas caused by overexpression of *Shh* differ from the follicular tumors observed in the β-catenin transgenic mice, the latter being those seen in families with colon cancer susceptibility (Cooper and Fechner, 1983). Likewise, patients with inadequate function of the Shh antagonist Ptc are not at increased risk for colon cancer. β-catenin stabilization and Shh therefore appear to play distinct roles in the growth of the epithelium.

Whether the hair spacing changes reflect a normal function of β-catenin or an aberrant function due to overexpression remains to be seen. Further information is needed about when and where β-*catenin* target genes are induced. One clue is the expression of the *Lef-1* transcription factor. *Lef-1* is found in the early hair placode and in proliferating cells of the growing hair, suggesting that these are possible sites of β-catenin activity (Zhou, et al., 1995). When the sites of normal β-*catenin* action become known, the relationship between *Shh* and β-*catenin* may be clearer. The effects of β-catenin stabilization on the spacing and number of placodes might be mediated by the ectopic induction of *Shh*. *Shh* expressed ectopically would induce new placodes and begin the growth process. One test of this hypothesis would be to overexpress activating components of the *Shh* signaling pathway in skin and examine them for similarities to the β-*catenin* phenotype.

Do the effects of mutant β-catenin implicate a Wnt signal in hair follicle morphogenesis? The strongest evidence in favor of a role for Wnt signaling in hair placode formation comes from analysis of mouse *Lef-1/TCF* mutants. These mutants lack whisker placodes and have abnormalities in body hair growth and differentiation. Tissue recombination studies show that *Lef-1* function is required in the mesoderm early in whisker development and in the epidermis later in the differentiating body hair (Kratochwil et al., 1996). A search for which Wnt may be

mediating these effects is currently underway. Wnt10b is produced in the hair follicle (St. Jacques et al., 1998), and Wnt7a is expressed in the early feather placode (Chuong, 1998), so they are both candidates. *Wnt3a* is normally expressed in the skin during embryogenesis and in epidermal cells of the follicle beginning to differentiate. However, overexpression of *Wnt3a* in the epidermis causes a shortened hair cycle and fragile hair shafts, but no disruption of the hair spacing, or tumor formation (Millar et al., 1998). Wnt molecules may have multiple functions during hair growth and development.

Work to Do

The common regulatory strategies used during organogenesis make studies of hair and feather development informative to many developmental and cancer biologists. Now we have some ideas about genes that control placode density and spacing and those that control growth and differentiation. Further testing of the conclusions gleaned from gain-of-function experiments awaits the development of tissue-specific, loss-of-function mutations. Key issues remain to be addressed, including the identification of the mesoderm-derived signals that induce the initial placode pattern, the interactions between *Shh* and *β-catenin* in growth control, and the molecular nature of the myriad epithelial–mesodermal interactions that control patterning and growth of the follicle. The segregation and maintenance of pluripotent stem cells, and cyclical organ regeneration—the hair cycle—are special aspects of skin development that offer exciting prospects. Understanding hair and feather development and regeneration, including the roles of β-catenin, could lead to new ideas about tumorigenesis and the regeneration of other appendages such as lung, liver, or pancreas. These really would be "hair-raising" results.

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