COMMENTARY
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WNTs: Multiple Genes, Multiple Functions

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The development of ectodermal appendages such as hair, feathers and teeth has long fascinated biologists, who have attempted to understand how cells of different types interact with each other to induce the coordinated changes in cell shape, proliferation, adhesion and movement required for the formation of these intricate three-dimensional structures. Classical experiments involving recombination of ectodermal and mesenchymal cells from different sources revealed that, for each of these types of appendage, reciprocal communication between the epithelium and mesenchyme is essential at each stage of development (Hardy, 1992; Jernvall and Thesleff, 2000). More recent molecular studies have identified some of the secreted signaling molecules involved in these processes, and have revealed that members of the same families of secreted factors regulate the initial stages of formation of all of these appendages (Jernvall and Thesleff, 2000; Millar, 2002). It is tempting to assume that these secreted factors play identical roles in the initiation of all appendage types. However, it seems more likely that subtle differences in the timing and location of signaling factor expression, and differences in the signaling family members utilized for different appendages, lay the groundwork for subsequent variations in development that ultimately lead to the obvious differences that we observe between feathers, teeth and hair, and even between different types of hair (for instance scalp, adnexal and body hair in humans).

WNT factors are among the most intensely investigated signaling molecules operating in appendage development. The WNT family comprises 18 known members in humans, and these bind to 10 known receptors (the Frizzled proteins) and several coreceptors (Wodarz and Nusse, 1998). WNT proteins activate multiple signaling pathways (Niehrs, 2001) displaying a versatility worthy of these most complex of organs. They play essential roles in many aspects of development, controlling cell proliferation, adhesion, fate, survival, movements and polarity (Huelsken and Birchmeier, 2001). Mutations in mouse *Wnt* genes cause diverse phenotypes, from defects in development of the brain and the posterior regions of the body, to alterations in limb polarity (Huelsken and Birchmeier, 2001).

In the best-studied WNT signaling pathway, the 'canonical' pathway, binding of secreted WNT protein to a Frizzled receptor activates an intracellular protein, Dishevelled, and causes stabilization of cytoplasmic β-catenin. β-catenin accumulates in the cytoplasm and nucleus and complexes with DNA binding factors of the LEF/TCF family, causing activation of WNT target gene transcription (Huelsken and Birchmeier, 2001). This pathway regulates cell fate and proliferation, and mutations in several pathway components have been implicated in the etiology of human tumors including colon cancer and melanoma (Polakis, 2000).

The first indication that the canonical WNT pathway might be active in appendage formation came from the finding that a loss of function mutation in the mouse *Lef1* gene causes defective development of hair, teeth and mammary glands (van Genderen *et al*, 1994). Subsequent analysis of the phenotypes caused by gain and loss of function mutations in WNT pathway members in chick and mouse (reviewed in Millar, 2002) and by ectopic expression of an endogenous canonical WNT inhibitor in transgenic mouse skin (Andl *et al*, 2002) confirmed the importance of this pathway in appendage development and in the etiology of pilomatricoma, a tumor of the hair follicle matrix.

An alternative noncanonical WNT pathway, activated by several *Wnts* that are endogenously expressed in developing hair and feather follicles, involves interaction of WNT with Frizzled and activation of Dishevelled, but does not require β-catenin or LEF/TCF, signaling instead through Jun amino-terminal kinase (JNK) (Niehrs, 2001). This pathway has been shown to regulate planar polarity in Drosophila and gastrulation movements in vertebrate embryos (Niehrs, 2001), and may play equally important roles in the developing skin and its appendages. Despite intensive study, the precise functions of both canonical and noncanonical WNT signals in developing appendages remain to be defined.

In an interesting paper in this issue, Chodankar *et al* (page 20) examine the function of one member of the *Wnt* family, *Wnt6*, in the developing feather follicle. Using short-term BrdU labeling, the authors demonstrate the presence of localized growth zones in the developing feather placode and bud. The locations of the growth zones shift as the buds develop, and, at each developmental stage, correlate with regions of the placode or bud that are undergoing expansion. These precisely demarcated areas of growth explain in part how the bud becomes polarized along the anterior-posterior and proximal-distal axes. Chodankar *et al* show that *Wnt6* is specifically expressed in regions of the placode and bud epithelia that are proliferating. To examine the effects of *Wnt6* on feather bud development, this gene was misexpressed from a replication competent avian sarcoma virus. This caused increased proliferation at the base, along the shaft, or at the tips of feather buds, resulting in local expansion of these regions. These results suggest that one of the functions of *Wnt6* is to regulate localized proliferation at early stages of feather bud development. The authors suggest that *Wnt6* may operate via the canonical WNT pathway, which is known to control proliferation in certain contexts. However, further experimentation will be required to test this hypothesis.

Chodankar *et al* show that several additional *Wnt* genes, two Frizzled receptor genes and the gene encoding secreted Frizzled related protein 2 (*sFrp2*), an endogenous WNT inhibitor, are expressed in developing feather follicles. These genes show expression patterns that overlap with, but are distinct from, that of *Wnt6*. Gain and loss of function experiments will be required to determine whether these genes also regulate proliferation, or whether they play some of the other roles that have been attributed to *Wnts*, such as controlling the fate, survival, adhesion, or movements of cells in developing follicles. Over- or ectopic expression of *Wnt7a* in embryonic chick skin and *Wnt3* in

transgenic mouse skin produces phenotypes that are distinct from that reported here for *Wnt6*: ectopic *Wnt7a* disrupts feather follicle polarity (Widelitz *et al*, 1999), while *Wnt3* alters the growth properties and differentiation of the hair shaft (Millar *et al*, 1999). Thus it is likely that *Wnt* genes perform multiple functions in appendages, and that these are specific for individual *Wnts* or subsets of *Wnts*. Such specificity may be mediated in part by differences in the affinities of WNT proteins for the various Frizzled receptors, particularly as members of the Frizzled family also show restricted and distinct patterns of expression within follicles. Other factors that may influence the effects of expression of a particular *Wnt* gene include the presence of WNT coreceptors, endogenous secreted inhibitors, and transcription partners for LEF/TCF factors. Most importantly, as mentioned above, WNT proteins can activate several different signaling pathways, resulting in quite different biological outcomes. For instance, *Wnt5a*, one of the genes whose expression is examined in the present paper, is known to be capable of activating a noncanonical pathway that regulates cell movements (Moon *et al*, 1993).

The mechanics of hair and feather follicle development show significant differences. For instance, after the initial formation of thickened placodes in chick embryo epithelium the feather rudiments bud outward from the skin and the epithelium at the base of the follicle does not invaginate into the dermis until the late long bud stage. In contrast, the developing mouse hair follicle epithelium invaginates into the underlying mesenchyme immediately after formation of the placode, and gradually folds around the dermal papilla. Interestingly, *Wnt6* and some of the other *Wnts* examined here differ in their expression patterns in developing feather and hair follicles. In the mouse, unlike the chick, *Wnt6* is expressed throughout the epithelium, and is not markedly up-regulated in hair follicles at early stages of their formation (Reddy *et al*, 2001). The expression of *Wnt5a* appears to initially overlap with that of *Wnt6* in the feather bud epithelium, whereas in the developing hair follicle *Wnt5a* first appears in the dermal condensate, and is apparent in the follicular epithelium starting at the bulbous peg stage (Reddy *et al*, 2001). Finally, *Wnt11* is expressed in distal feather bud mesenchyme and in interbud epithelium in the chick, whereas in the mouse embryo it is primarily expressed in the dermis, and appears in follicular epithelial cells at later stages of follicle development (Reddy *et al*, 2001).

These differences in *Wnt* expression patterns in mammalian and avian skin might be a consequence of differences in the developmental mechanics of feather and hair formation. However, a more interesting, and perhaps more likely, possibility is that differences in the mechanics of hair and feather follicle development are controlled by variations in the timing and location of expression of the various *Wnt* genes, as these have the potential to regulate cellular proliferation, fate, survival, movement and adhesion. Thus a careful comparison of gene expression in different species, and in different types of follicle within a species, is likely to provide us with important clues in our continuing hunt for the follicle's molecular treasures.

REFERENCES

Andl T, Reddy ST, Gaddapara T, Millar SE: WNT Signals Are Required for the Initiation of Hair Follicle Development. *Dev Cell* 2:643–653, 2002

van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R: Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev* 8:2691–2703, 1994

Hardy MH: The secret life of the hair follicle. *Trends Genet* 8:55–60, 1992

Huelsken J, Birchmeier W: New aspects of Wnt signaling pathways in higher vertebrates. *Curr Opin Genet Dev* 11:547–553, 2001

Jernvall J, Thesleff I: Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mech Dev* 92:19–29, 2000

Millar SE: Molecular mechanisms regulating hair follicle development. *J Invest Dermatol* 118:216–225, 2002

Millar SE, Willert K, Salinas PC, Roelink H, Nusse R, Sussman DJ, Barsh GS: WNT Signaling in the Control of Hair Growth and Structure. *Dev Biol*, 207:133–149, 1999

Moon RT, Campbell RM, Christian JL, McGrew LL, Shih J, Fraser S: Xwnt-5A, a maternal Wnt that affects morphogenetic movements after overexpression in embryos of Xenopus laevis. *Development* 119:97–111, 1993

Niehrs C: Solving a sticky problem. *Nature* 413:787–788, 2001

Polakis P: Wnt signaling and cancer. *Genes Dev* 14:1837–51, 2000

Reddy S, Andl T, Bagasra A, Lu MM, Epstein DJ, Morrisey EE, Millar SE: Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. *Mech Dev* 107:69–82, 2001

Widelitz RB, Jiang TX, Chen CW, Stott NS, Chuong CM: Wnt-7a in feather morphogenesis: involvement of anterior-posterior asymmetry and proximal-distal elongation demonstrated with an in vitro reconstitution model. *Development* 126:2577–2587, 1999

Wodarz A, Nusse R: Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 14:59–88, 1998