Molecular Mechanisms Regulating Hair Follicle Development

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Clinical conditions causing hair loss, such as androgenetic alopecia, alopecia areata, and scarring alopecia, can be psychologically devastating to individuals and are the target of a multimillion dollar pharmaceutical industry. The importance of the hair follicle in skin biology, however, does not rest solely with its ability to produce hair. Hair follicles are self-renewing and contain reservoirs of multipotent stem cells that are capable of regenerating the epidermis and are thought to be utilized in wound healing. Hair follicles are also the sites of origin of many neoplasias, including some basal cell carcinomas and pilomatricoma. These diseases result from inappropriate activation of signaling pathways that regulate hair follicle morphogenesis. Identification of the signaling molecules and pathways operating in developing and postnatal, cycling, hair follicles is therefore vital to our understanding of pathogenic states in the skin and may ultimately permit the development of novel therapies for skin tumors as well as for hair loss disease. The purpose of this review is to summarize recent progress in our understanding of the molecular mechanisms regulating hair follicle formation, and to discuss ways in which this information may eventually be utilized in the clinic.* ***Key words: dermis/epidermis/morphogenesis/signaling/skin. J Invest Dermatol 118:216–225, 2002***

STRUCTURE AND PROPERTIES OF THE HAIR FOLLICLE

The mature hair follicle is a complex structure, composed of several concentric cylinders of epithelial cells, known as root sheaths, which surround the hair shaft (Sperling, 1991) (**Fig 1**). Although it is largely epithelial in origin, the follicle contains at its base a ball of specialized dermal cells, the dermal papilla, which play a crucial part in the regulation of successive cycles of postnatal hair growth (**Fig 2**). At the onset of phases of hair growth, signals from the dermal papilla are thought to instruct epithelial stem cells residing in the bulge region of the follicle to divide transiently (Oliver and Jahoda, 1988; Cotsarelis *et al*, 1990; Wilson *et al*, 1994; Lyle *et al*, 1998). Stem cell progeny migrate to the base of the follicle, where they surround the dermal papilla, forming the hair matrix (Oshima *et al*, 2001; Taylor *et al*, 2000). In response to further signals from the dermal papilla, matrix cells proliferate and begin the process of terminal differentiation, moving upward in the follicle and forming the hair shaft and inner root sheath (Oliver and Jahoda, 1988; Taylor *et al*, 2000; Oshima *et al*, 2001). Pigmentation of the hair results from the activity of melanocytes, which reside in the hair follicle bulb and deposit pigment granules into the hair shaft as it forms. Periods of hair growth are followed by a regression phase, when the lower part of the follicle undergoes programmed cell death (Cotsarelis, 1997), and a resting phase, before onset of a new growth phase (**Fig 2**). Cyclical growth of hair continues throughout postnatal life, and allows the follicle to remodel itself. This is particularly evident in the response of hair follicles to androgens, which cause enlargement of beard hair follicles in adolescent boys, and miniaturization of scalp hair follicles in men with androgenetic alopecia (Hamilton, 1942).

Manuscript received June 19, 2001; revised September 21, 2001; accepted for publication October 15, 2001.

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COMMUNICATION BETWEEN CELLS OF DIFFERENT TYPES IS REQUIRED FOR HAIR FOLLICLE FORMATION

The formation of hair follicles occurs during embryogenesis and relies on a series of signals sent between dermal cells and overlying surface epithelial cells that cause fate changes in both cell populations, ultimately resulting in differentiation of the hair shaft, root sheaths, and dermal papilla (Hardy, 1992). The existence of these signals was revealed by experiments performed as long ago as the 1950s, in which dermis and epidermis of different origins were recombined at different embryologic stages. These experiments utilized mouse and chick skin, and took advantage of similarities in the mechanisms regulating the early steps of hair and feather development. It was found that an initial signal arising in the dermis (the ''first dermal signal'') causes the formation of regularly spaced thickenings in the epidermis, known as placodes (Hardy, 1992) (**Fig 3**). An ''epithelial signal'' from the placode causes the clustering of a group of underlying cells in the mesenchyme, forming a ''dermal condensate''. In response to a ''second dermal message'' from the dermal condensate, the epithelial placode cells proliferate and invade the dermis, eventually surrounding the dermal condensate, which develops into the hair follicle dermal papilla (Hardy, 1992). Further proliferation and differentiation of the epithelial cells results in the formation of the inner root sheath and hair shaft of the mature follicle, processes that are likely to require lateral communication between epithelial cells (Millar *et al*, 1999; Lin *et al*, 2000).

The finding that communication between different types of cells is critical for hair follicle development implies that intercellular signaling molecules play key roles in this process. A variety of approaches have been taken to identify these molecules, their effectors, and their downstream targets, including surveys of the

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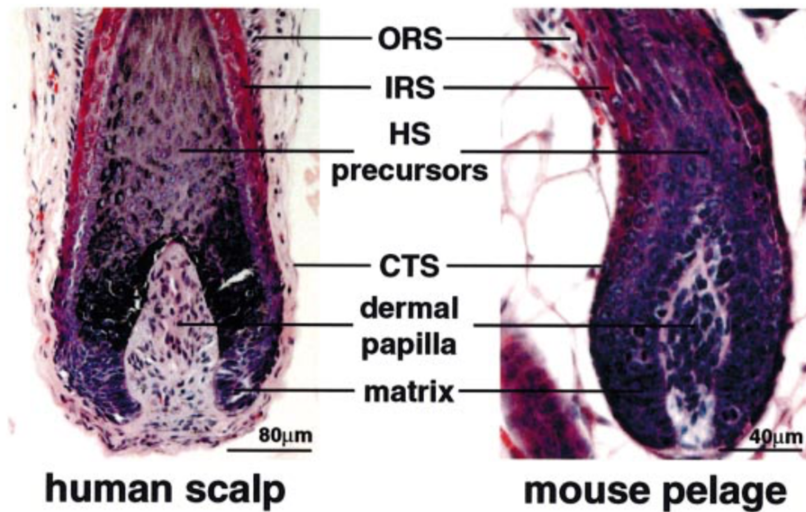

Figure 1. Comparison of the structure of the hair bulb in human scalp and mouse pelage. Paraffin sections were stained with hematoxylin and eosin and photographed at magnifications of ×10 (human) and ×20 (mouse). IRS, inner root sheath; ORS, outer root sheath; CTS, connective tissue sheath; HS, hair shaft.

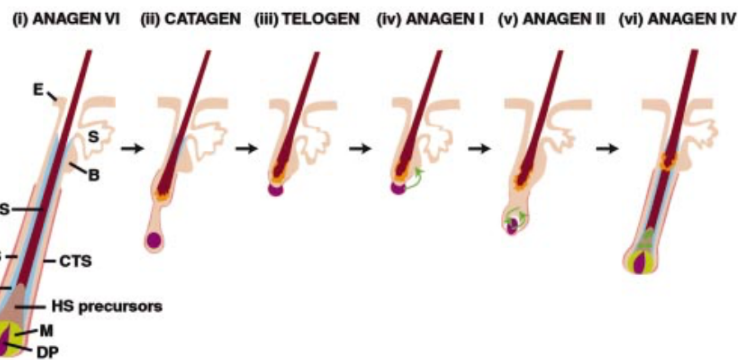

Figure 2. Schematic depiction of the hair growth cycle. (*i*) During anagen, matrix cells proliferate and differentiate to form the inner root sheath and hair shaft. (*ii*) During catagen the lower two-thirds of the follicle undergo programmed cell death. (*iii*) The follicle enters a resting phase, telogen. (*iv*) At the onset of a new cycle of hair growth, follicular epithelial stem cells are stimulated to divide by signals from the dermal papilla (*green arrow*). (*v*) During anagen signals from the dermal papilla stimulate the division of matrix cells and the inductive properties of the dermal papilla are maintained by signals from the follicular epithelium (*green arrows*). (*vi*) Differentiation of matrix cells into hair shaft and inner root sheath may involve lateral signaling between epithelial cells (*green arrows*). IRS, inner root sheath; E, epidermis; HS, hair shaft; ORS, outer root sheath; DP, dermal papilla; M, matrix; CTS, connective tissue sheath; B, bulge; S, sebaceous gland.

expression patterns of families of candidate signaling factors, the analysis of transgenic and knockout mice carrying mutations in candidate genes, and analysis of the effects of introducing candidate molecules through bead implantation into the skin. Most of these studies have been carried out in animal systems, particularly mouse and chick. These are not ideal systems for studying all aspects of human hair follicle biology, for instance mouse and human follicles show differing responses to androgens. Descriptive analyses of gene expression patterns in human skin, where available, however, suggest that the molecules regulating the basic mechanisms of follicle development are similar in human, mouse, and chick (Holbrook *et al*, 1993; Kaplan and Holbrook, 1994). Importantly, human families carrying mutations in genes controlling hair follicle development show similar phenotypes to the corresponding mouse mutants (Cachon-Gonzalez *et al*, 1994; Nehls *et al*, 1994; Segre *et al*, 1995; Brissette *et al*, 1996; Hahn *et al*, 1996; Johnson *et al*, 1996; Kere *et al*, 1996; Unden *et al*, 1996; Ferguson *et al*, 1997; Srivastava

hair follicle formation remain unknown or partially characterized, recent studies have led to an explosion of information about signaling in developing hair follicles, providing exciting opportunities for therapeutic innovation.

THE FIRST DERMAL SIGNAL

It is well established from experiments in the mouse and chick, that dermis from body regions that will eventually develop hair or feathers when combined with epidermis from non-hair bearing regions, will direct the formation of appendages of the size and spacing characteristic of the region from which the dermis was derived (Hardy, 1992). Thus, in humans, one would expect that scalp dermis is able to induce the formation of large hair follicles that produce thick, long hair, whereas dermis from the forearm directs the formation of smaller follicles. Consistent with this, dermal sheath dissected from a male human scalp was able to induce the formation of thick, darkly pigmented hair when implanted into

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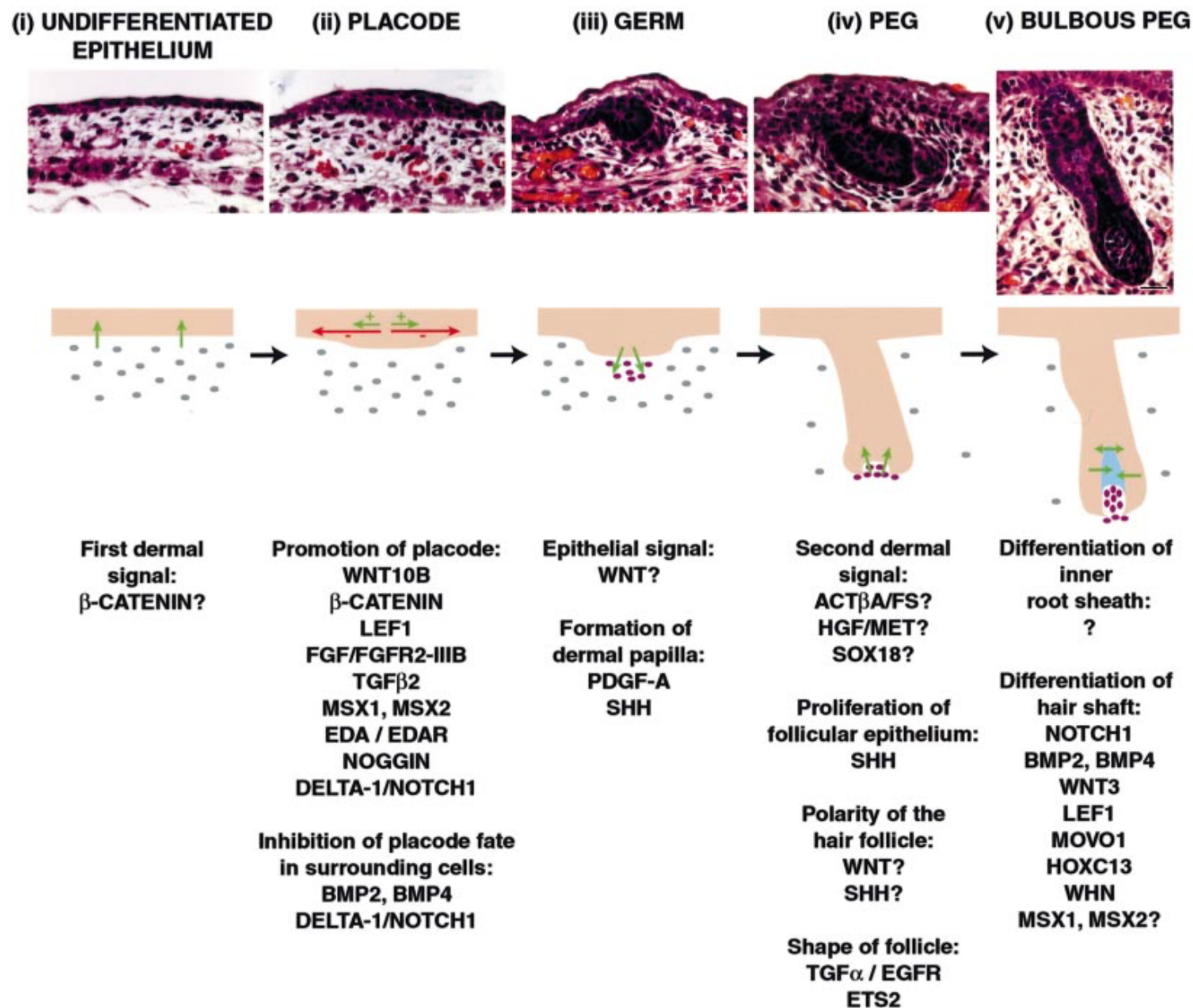

Figure 3. The events of hair follicle morphogenesis. The upper panels show paraffin sections of primary mouse hair follicles at embryonic days 14.5 (undifferentiated epithelium and placode), 15.5 (germ and peg), and 18.5 (bulbous peg), stained with hematoxylin and eosin. The middle panels show these same developmental stages represented schematically, with intercellular signals indicated by colored arrows, and candidate molecules important for each stage listed below (see text for references). (*i*) The initial signal directing hair follicle formation arises in the mesenchyme (gray dots) and instructs the overlying epithelium (pink stripe) to thicken, forming a placode. (*ii*) Placode formation is facilitated by promoting signals (+), shown as *green arrows*, and prevented in neighboring epithelial cells by inhibitory signals (–), shown as *red arrows*. (*iii*) Signals from the epithelium induce the clustering of mesenchymal cells to form a dermal condensate (*purple dots*). (*iv*) The dermal condensate signals to the follicular epithelium to proliferate and grow down into the dermis. (*v*) The dermal condensate becomes enveloped by follicular epithelial cells to form the dermal papilla. Differentiation of the inner root sheath (*blue*) may be regulated in part by lateral signaling between epithelial cells (*green arrows*). Photographs in each of the upper panels were taken at the same magnification. *Scale bar:* 20 μm.

determining the identity of the ''first dermal signal'' is likely to be of key importance. Currently, however, this signal has not been characterized, and its mode of action remains a subject for speculation.

In one model to explain how the dermal signal operates, the spacing and size of placodes are regulated by an intrinsically periodic dermal signal, which varies in character in different body regions. To test this hypothesis, Jiang *et al* (1999) marked dermal condensates in chick skin by injection of the lipophilic dye, DiI, and then dissociated the dermis and epidermis into single cell suspensions. When these cells were recombined, a regular array of feather placodes and dermal condensates was established. Labeled

placodes, and arguing against the idea of a periodic dermal signal. In an alternative model, the dermal signal occurs uniformly within each body region and triggers the activation of promoters and repressors of follicle fate that then compete with one another, resulting in the establishment of a regular array of follicles (Slack, 1991; Barsh, 1999). Differences in the levels of promoter and repressor activation might then account for regional differences in the size and spacing of follicles. Consistent with this model, several positive and negative regulators of hair follicle fate are initially expressed uniformly in the epidermis and subsequently become localized to placodes (see below).

A first clue to the molecular nature of the dermal signal has come

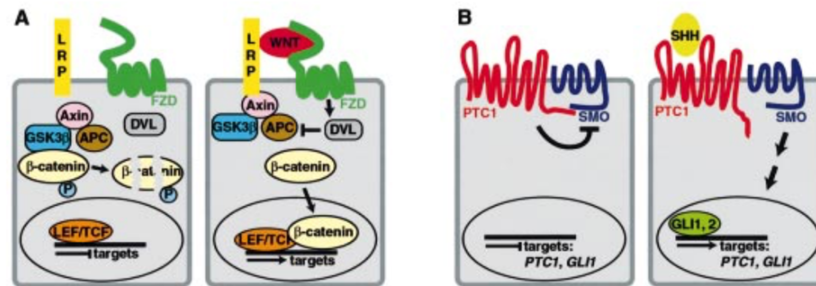

Figure 4. Schematic depictions of the WNT and Sonic hedgehog (SHH) signaling pathways. (*A*) The WNT signaling pathway. In the absence of a WNT signal, cytoplasmic β-catenin is phosphorylated and targeted for degradation by a complex of proteins, including axin, adenomatous polyposis coli tumor suppressor protein (APC) and glycogen synthase 3-β (GSK3-β). In the presence of a WNT signal the degradation machinery is inhibited and β-catenin translocates to the nucleus where it forms a transcription complex with DNA binding factors of the lymphoid enhancer-binding factor/T cell factor (LEF/TCF) family, and activates transcription of target genes. (*B*) The SHH signaling pathway. In the absence of an SHH signal, activity of the smoothened (SMO) protein is inhibited by the actions of the SHH receptor Patched 1 (PTC1). In the presence of SHH, the repression of SMO is lifted, and target genes, including *PTC1* and *GLI1*, are transcribed through the actions of the transcription factors GLI1 and GLI2.

over short distances to control cell fate in many organ systems, degradation of cytoplasmic β-catenin is inhibited. In these circumstances β-catenin accumulates in the cytoplasm and translocates to the nucleus where it forms transcriptionally active complexes with members of the lymphoid enhancer-binding factor/T cell factor (LEF/TCF) family of DNA binding factors (**Fig 4*A***) (reviewed in Wodarz and Nusse, 1998). In the chick, nuclear β-catenin is found transiently in the dense dermis underlying the feather tract 2 d before the appearance of molecular and morphologic signs of placode development (Noramly *et al*, 1999). Consistent with this, *Lef1* is expressed in the mesenchyme of the mouse vibrissa pad prior to vibrissa follicle development, and initiation of vibrissa follicle development is dependent on this expression (Kratochwil *et al*, 1996). Interestingly, development of dorsal feather inducing dermis has recently been found to be dependent on a signal from the dorsal neural tube, which can be substituted by *Wnt*1 (Olivera-Martinez *et al*, 2001). These findings suggest that activation of the WNT signaling pathway in the dermis may be involved in establishing the first dermal signal. The appearance of nuclear β-catenin is uniform within the dense dermis (Noramly *et al*, 1999), consistent with the hypothesis that the "first dermal signal" is uniform rather than periodically localized.

FORMATION OF PLACODES: A COMPETITION BETWEEN PLACODE PROMOTERS AND PLACODE REPRESSORS

Descriptive and functional studies in mouse and chick have revealed that in response to the first dermal signal, members of several classes of signaling molecules are expressed in the follicular epithelium. These include both promoters and repressors of placode fate. Several lines of evidence suggest that WNT paracrine signaling molecules act early in the process of follicle formation to promote placode fate. In the mouse embryo, *Wnt10b* is initially expressed uniformly in the epidermis and is markedly upregulated in placodes (St-Jacques *et al*, 1998; Reddy *et al*, 2001) and *Wnt7a* shows a similar pattern of expression in embryonic chick skin (Widelitz *et al*, 1999). In the chick, 1 d after the appearance of nuclear β-catenin in the dense dermis, nuclear β-catenin is detected in the overlying epithelium, indicating that the WNT signaling pathway is activated in the epithelium (Noramly *et al*, 1999). Like expression of mouse *Wnt10b* and chick *Wnt7a*, nuclear β-catenin becomes gradually elevated in regions destined to form placodes (Noramly *et al*, 1999). DasGupta and Fuchs (1999) have generated transgenic mice bearing a TOPGAL reporter gene that is responsive

and dermal condensates, providing an indication that the WNT pathway is active in both epithelial and mesenchymal components of developing follicles (DasGupta and Fuchs, 1999). Expression of a stabilized form of β-catenin protein in chick or mouse epidermis causes the formation of ectopic follicles, indicating that activation of the WNT signaling pathway in the epithelium is sufficient to direct follicle development (Gat *et al*, 1998; Noramly *et al*, 1999; Widelitz *et al*, 2000). Conversely, loss of function of the β-catenin gene in mouse epidermis results in a failure of placode development (Huelsken *et al*, 2001), and a null mutation of the mouse gene encoding the WNT effector protein LEF1 causes failure of formation of vibrissae and two-thirds of the body hair follicles (van Genderen *et al*, 1994; Kratochwil *et al*, 1996).

Elegant tissue recombination experiments performed by Kratochwil *et al* (1996) demonstrated that expression of *Lef1* in the mesenchyme is necessary for the initiation of mouse vibrissa follicle development, and that transient expression of *Lef1* in the epithelium is required for the completion of morphogenesis. Pelage hair follicles developed normally in the absence of mesenchymal *Lef1*, but formed in reduced numbers and with less pronounced keratinization in the absence of epithelial *Lef1*. These results suggest that WNT signaling is required at several different stages in the development of vibrissae and pelage hair follicles, and that there may be partial functional redundancy of LEF1 with other LEF/TCF family members. Three other members of this gene family have been identified: *Tcf1*, *Tcf3*, and *Tcf4*. *Tcf1* is reportedly not expressed in keratinocytes (Zhou *et al*, 1995); however, its expression in the dermis preceding hair follicle development has not been examined. *Tcf3* is known to be expressed in the skin and hair follicles, although the TCF3 protein is generally associated with repression of the canonical WNT signaling pathway (Barker *et al*, 1999; Merrill *et al*, 2001). TCF4 protein is apparently not expressed in human keratinocytes or hair follicles in the second trimester (Barker *et al*, 1999); however, expression of TCF4 in the dermis and epithelium at earlier stages of human hair follicle development has not been carefully analyzed. Close inspection of published *in situ* hybridization data reveals expression of *Tcf4* in the skin and vibrissa plate of the mouse embryo at embryonic day (E)13.5 (Korinek *et al*, 1998) and in vibrissa follicles at E15.5 and E18 (Lee *et al*, 1999). It is likely that further analysis of *Tcf1* and *Tcf4* expression patterns in skin, vibrissa, and hair follicles will reveal which of these genes might substitute for *Lef1* at certain stages of follicle development.

In addition to ectopic follicles, expression of stabilized β-catenin in mouse or chick causes follicle-based tumors (Gat *et al*, 1998;

molecular link between the early events of follicle formation and tumorigenesis.

Fibroblast growth factors (Fs) and FGF receptor genes are expressed at early stages of follicle development and also appear to promote follicle formation, as exogenous FGF molecules induce ectopic follicles in wild-type chick embryos, and feather buds in *scaleless* (*sc/sc*) embryos that otherwise fail to develop feathers as a result of an ectodermal defect (Chuong *et al*, 1996; Song *et al*, 1996; Widelitz *et al*, 1996; Jung *et al*, 1998). Conversely, a loss of function mutation in the mouse gene encoding fibroblast growth receptor 2-IIIB causes defective development of the skin and hair follicles (Revest *et al*, 2001). Overexpression of FGF4 in embryonic chick skin alters the pattern of β-catenin mRNA expression suggesting a possible role for FGF in elevating β-catenin expression in placodes (Widelitz *et al*, 2000).

The gene encoding transforming growth factor-β (TGF-β2) is expressed in both the placode and the dermal condensate (Ting-Berreth and Chuong, 1996a; Ting-Berreth and Chuong, 1996b), and TGF-β2-soaked beads can induce the formation of dermal papillae in chick embryo mesenchyme that has been stripped of epithelium, and hair follicles in mouse embryo skin explants (Ting-Berreth and Chuong, 1996b; Foitzik *et al*, 1999), suggesting TGF-β2 as another promoter of follicle fate. Consistent with this, mice lacking a functional *Tgfβ2* gene have reduced numbers of hair follicles and a delay in follicular morphogenesis (Foitzik *et al*, 1999). The homeobox-containing genes *Msx1* and *Msx2* are expressed in placodes (Noveen *et al*, 1995), and mutant mice lacking both of these genes have reduced numbers of hair follicles (Satokata *et al*, 2000), indicating that the MSX1 and MSX2 transcription factors also play important parts in promoting placode fate.

Recently, ectodysplasin (EDA), a molecule related to tumor necrosis factor, and its receptor ectodysplasia receptor (EDAR) have been identified as essential factors for initiating the development of hair follicles on the basis of their mutant phenotypes in humans and mice (reviewed in Barsh, 1999). The *EDA* gene is mutated in human X-linked anhidrotic ectodermal dysplasia, and in the *Tabby* mouse, causing decreased numbers of hair follicles, and defects of the teeth and sweat glands (Kere *et al*, 1996; Ferguson *et al*, 1997; Srivastava *et al*, 1997). The *EDAR* gene is mutated in human autosomal recessive and dominant hypohidrotic ectodermal dysplasias (Monreal *et al*, 1999) and in the *downless* mouse (Headon and Overbeek, 1999), causing identical phenotypes to those resulting from *EDA* mutations. The mouse *Edar* mRNA is expressed ubiquitously in the epithelium prior to placode formation, and then becomes restricted to placodes (Headon and Overbeek, 1999), whereas the *Eda* mRNA is ubiquitously expressed even after placode formation (Srivastava *et al*, 1997; Mikkola *et al*, 1999). Based on its similarity to the tumor necrosis factor family, EDA is likely to be cleaved from the membrane and to be capable of diffusing to sites of EDAR expression (Headon and Overbeek, 1999). Loss of EDA/EDAR signaling in mutant mice affects the formation only of specific subtypes of hair follicles (Headon and Overbeek, 1999). Two other EDAR family members, TROY and X-linked ectodysplasin-A2 receptor (XEDAR), are expressed in developing hair follicles (Kojima *et al*, 2000; Yan *et al*, 2000) and may substitute for EDAR in unaffected hair types; alternatively, a different signaling pathway may be utilized.

In contrast to EDA and EDAR, members of the bone morphogenic protein (BMP) family of secreted signaling molecules appear to act as inhibitors of follicle formation. *Bmp2* is expressed diffusely in the ectoderm, but then locates at an early stage to the preplacode epithelium and underlying mesenchyme (Noramly and Morgan, 1998). *Bmp7* is expressed in placodes, and *Bmp4* is expressed in the prefollicle mesenchyme (Jung *et al*, 1998; Noramly and Morgan, 1998; Huelsken *et al*, 2001). Ectopic expression of *Bmp2* or *Bmp4* suppresses the formation of feather buds in chick embryos (Jung *et al*, 1998; Noramly and Morgan, 1998). In the both for promoting the placode and for lateral inhibition of placode fate in surrounding cells (Barsh, 1999; Headon and Overbeek, 1999). Expression of β-catenin in the epithelium is also necessary for the expression of *Bmp4* and *Shh*, as well as *Bmp2* and *Bmp7*, indicating that WNT signaling in the epithelium is also required at a very early stage of morphogenesis (Huelsken *et al*, 2001). *Edar* is expressed in the absence of epithelial β-catenin (Huelsken *et al*, 2001), suggesting that *Edar* expression is either regulated independently of epithelial β-catenin, or lies upstream of activation of WNT signaling in the epithelium.

The genes encoding several secreted molecules capable of inhibiting BMP action, including Noggin, Follistatin (FS), and Gremlin, are expressed in developing follicles (Feijen *et al*, 1994; Roberts and Barth, 1994; Noramly and Morgan, 1998; Jiang *et al*, 1999; Merino *et al*, 1999; Patel *et al*, 1999; Ohyama *et al*, 2001). These are thought to negate BMP action within the follicle, but, in contrast to BMP, may not diffuse into the interfollicular regions. Support for this hypothesis comes from the phenotype of mice lacking *Noggin*, which have fewer hair follicles than normal and retarded follicular development (Botchkarev *et al*, 1999). Expression of *Lef1* is reduced in *Noggin*-null mice, suggesting that *Lef1* expression may be repressed by BMP (Botchkarev *et al*, 1999). Conversely, ectopic expression of *Noggin* in chick or mouse embryonic skin causes enlarged and ectopic follicles (Noramly and Morgan, 1998; Botchkarev *et al*, 1999; Jiang *et al*, 1999). The Notch pathway also appears to play a part in determining the follicular pattern. The Notch ligand *Delta1* is normally expressed in the mesenchyme underlying the placode (Crowe *et al*, 1998; Crowe and Niswander, 1998; Powell *et al*, 1998; Viallet *et al*, 1998), and when misexpressed in a small area of epithelium promotes expression of *Notch1* and accelerates placode formation, while suppressing placode formation in surrounding cells (Crowe *et al*, 1998; Viallet *et al*, 1998). Restriction of Delta-1 expression to the prefollicle may be controlled by FGF, as in *scaleless* chick mutants *Delta1* is ubiquitously expressed in the mesenchyme, but becomes localized after addition of FGF (Viallet *et al*, 1998).

In summary, the formation of placodes in response to the first dermal signal involves activation of EDA/EDAR signaling in the epithelium, followed by epithelial WNT signaling, and subsequent activation of BMP signaling (**Fig 3**). The actions of EDA/EDAR and WNT promote placode formation, whereas BMP signaling represses placode fate in adjacent skin. FGF signaling promotes placode fate, and regulates expression of *Delta1*. TGF-β2 and MSX factors also promote piacorse fate, but their interactions with other factors regulating hair follicle development have not yet been established.

INDUCTION OF THE DERMAL CONDENSATE: THE FIRST EPITHELIAL SIGNAL

WNT signaling is likely to be required for induction of the dermal condensate. Evidence for this is that the WNT-responsive TOPGAL reporter gene is expressed in the dermal condensate as well as in follicular epithelium (DasGupta and Fuchs, 1999), and the dermal condensate fails to develop in the absence of epithelial β-catenin (Huelsken *et al*, 2001).

Platelet-derived growth factor-A is expressed in the placode, whereas its receptor is expressed in the dermal condensate (Karlsson *et al*, 1999). Mice lacking platelet-derived growth factor-A have small dermal papillae, dermal sheath abnormalities, and thin hair, compared with wild-type siblings, suggesting that platelet-derived growth factor-A is also required for normal cross-talk between the follicle epithelium and its mesenchyme (Karlsson *et al*, 1999).

Another secreted protein present in the follicular placode that plays a major part in epithelial–mesenchymal signaling is Sonic hedgehog (SHH) (**Fig 4*B***) (Bitgood and McMahon, 1995; Iseki *et al*, 1996). In mice lacking SHH, hair follicle formation is initiated

subsequent signaling from the epithelium to both epithelial and mesenchymal cells, regulating proliferation and further downgrowth of the follicular epithelium and development of the dermal papilla (St-Jacques *et al*, 1998; Chiang *et al*, 1999; Karlsson *et al*, 1999). *Shh* expression is absent from the follicles of mice lacking epithelial β-catenin, indicating that *Shh* lies downstream of WNT signaling in hair follicle development (Huelsken *et al*, 2001). Hair keratin genes are expressed in $Shh^{-/-}$ hair follicles, indicating that SHH is not required for the initiation of hair shaft differentiation (St-Jacques *et al*, 1998; Chiang *et al*, 1999). The genes encoding Patched1 (PTC1), a receptor for SHH, and GLI1, a transcriptional effector of SHH signaling, are expressed in follicular epithelium and in the dermal condensate, consistent with the idea that SHH signals are required for the development of both components of the follicle (Dahmane *et al*, 1997; Platt *et al*, 1997; Ghali *et al*, 1999).

Mutations in *PTC1* play a causative role in the etiology of the majority of human basal cell carcinomas (BCC) (Hahn *et al*, 1996; Johnson *et al*, 1996; Unden *et al*, 1996), tumors that have several characteristics in common with immature hair follicles, including similar histology, ultrastructure, and patterns of keratin gene expression (Kumakiri and Hashimoto, 1978; Markey *et al*, 1992; Jih *et al*, 1999). These findings have led to intense interest in the regulation of the SHH signaling pathway in skin and hair, and recognition of the central part played by hair follicle signaling pathways in skin tumorigenesis (Gailani *et al*, 1996; Johnson *et al*, 1996). Identification of the downstream genes regulated by SHH signaling in hair follicles and BCC is of particular interest, as these are potential targets for novel BCC therapies. A *Wnt* family member, *Wnt5a*, is expressed in the dermal condensate of developing hair follicles, and is absent from these cells in $Shh^{-/-}$ embryos, suggesting it as a target of SHH signaling in hair follicles (Reddy *et al*, 2001). Interestingly, *Wnt5a* message is upregulated in a *Xenopus* model for BCC, and in human BCC samples (Bonifas *et al*, 2001; Mullor *et al*, 2001).

Tgfβ2 has been suggested to be a target of SHH signaling in follicles as its expression domain is widened in response to ectopic expression of SHH in embryonic chick skin (Ting-Berreth and Chuong, 1996a); however, normal levels of *Tgfβ2* message were detected in the hair follicles of mice lacking SHH (St-Jacques *et al*, 1998). Loss of function mutations in the *Tgfβ2* gene result in the development of reduced numbers of hair follicles (Foitzik *et al*, 1999) (see above), indicating that, whatever its relationship to *Shh*, this gene plays critical roles in hair follicle morphogenesis. The neurotrophin receptors TrkC and p75 neurotrophin receptor (p75NTR) are expressed in developing hair follicles in the placode and dermal condensate, respectively, and loss of function mutations in the *TrkC* and p75NTR genes, as well as in the gene encoding neurotrophin 3 (NT-3), affect the rate of hair follicle morphogenesis, suggesting that neurotrophins may also be involved at early steps of this process (Botchkarev *et al*, 1998, 1999a, b).

In summary, WNT and platelet-derived growth factor-A molecules are strong candidates as components of the first epithelial signal inducing formation of the dermal condensate. SHH acts later in follicular morphogenesis, is dependent on WNT signaling and is required for proliferation of follicular epithelium and development of the dermal condensate into a dermal papilla (**Fig 3**).

EPITHELIAL PROLIFERATION AND DOWNGROWTH: THE SECOND DERMAL SIGNAL

The "second dermal signal" regulating proliferation and downgrowth of the follicular epithelium is likely to be activated by SHH, as significant downgrowth fails to occur in SHH-null mice (St-Jacques *et al*, 1998; Chiang *et al*, 1999); however the nature of this signal is not known. One possible candidate is activin βA (ActβA), a secreted signaling molecule expressed in the dermal condensate (Feijen *et al*, 1994) (Roberts and Barth, 1994). Mice lacking ActβA

pattern complementary to that of ActβA (Feijen *et al*, 1994; Roberts and Barth, 1994), and mice lacking FS have thin, inappropriately oriented vibrissae due to defects in vibrissa follicle development (Matzuk *et al*, 1995b). Hepatocyte growth factor/scatter factor is expressed in the dermal condensate and its receptor, Met, is expressed in the follicular epithelium (Lindner *et al*, 2000). Mice overexpressing hepatocyte growth factor have increased numbers of hair follicles and accelerated hair follicle development, suggesting a possible role for this molecule in signaling between the follicular mesenchyme and epithelium (Lindner *et al*, 2000).

A member of the Sry-type high mobility group box (SOX) transcription factor family, SOX18, is expressed in the dermal condensate, and mice homozygous for a semidominant mutation in this gene lack several subtypes of hair, suggesting a role for SOX family members in the development or function of the dermal condensate (Pennisi *et al*, 2000a, b). Analyses of conditional mutant mice have revealed that expression of β1 integrin in the epithelium is required for remodeling the basement membrane and follicular downgrowth (Brakebusch *et al*, 2000; Raghavan *et al*, 2000), and that α-catenin is also required for normal follicular morphogenesis (Vasioukhin *et al*, 2001), revealing essential roles for adhesion molecules in this process.

DIFFERENTIATION OF THE INNER ROOT SHEATH AND HAIR SHAFT

As the hair follicle bulb appears (bulbous peg stage; **Figs 1**, **3**), at least seven different epithelial cell layers constituting the components of the mature hair follicle are formed (Sperling, 1991). Recent studies have begun to reveal some information about the genes responsible for determining and maintaining the phenotypes of particular layers within the highly organized hair follicle structure. The genes encoding Notch1, a membrane protein involved in determining cell fate through cell–cell interactions and intracellular signal transduction, and its ligands Serrate1 and Serrate2, are expressed in matrix cells destined to form the inner root sheath and hair shaft (Kopan and Weintraub, 1993; Powell *et al*, 1998; Favier *et al*, 2000), and expression of activated *Notch1* in hair shaft precursor cells under the control of a mouse hair keratin A1 promoter causes failure of differentiation of the hair shaft medulla (Lin *et al*, 2000); this suggests that Notch1 is one of the factors responsible for controlling the phenotype of keratinocytes as they leave the bulb matrix and differentiate into specific cell types of the follicle.

In mature hair follicles, *Bmp4* is expressed in the dermal papilla and both *Bmp2* and *Bmp4* are expressed in hair shaft precursor cells (Wilson *et al*, 1999; Kulessa *et al*, 2000). Ectopic expression of *Bmp4* in the hair follicle outer root sheath in transgenic mice inhibits the proliferation of matrix cells and activates hair keratin gene expression in the outer root sheath (Blessing *et al*, 1993), suggesting that BMP signaling is important for hair shaft differentiation. This hypothesis is supported by the phenotype of transgenic mice expressing the BMP inhibitor Noggin in matrix cells of the hair bulb under the control of an *Msx2* promoter. Expression of Noggin causes severe defects in differentiation of the hair shaft cortex and cuticle, loss of hair shaft differentiation markers, including trichohyalin and acidic hair keratins, and inhibition of the expression of several transcription factors normally expressed in mature follicles (see below) (Kulessa *et al*, 2000).

Several lines of evidence suggest a role for WNT signaling in regulating differentiation of the hair shaft. First, expression of the WNT-responsive TOPGAL reporter gene appears in hair shaft precursor cells as they begin the process of terminal differentiation (DasGupta and Fuchs, 1999); these cells also express LEF1 protein (DasGupta and Fuchs, 1999), and Dishevelled 2 (Millar *et al*, 1999), a component of the WNT signaling pathway, whereas adjacent cells express *Wnt3* (Millar *et al*, 1999). Ectopic expression of *Wnt3*

hair shafts that are made appear poorly keratinized (Kratochwil *et al*, 1996). The regulatory regions of hair shaft keratin genes contain binding sites for LEF1 (Zhou *et al*, 1995). Mutation of the LEF1 site in the promoter of the wool keratin intermediate filament gene *K2.10* decreased promoter activity in the hair follicles of transgenic mice, suggesting that WNT signaling might regulate expression of hair shaft intermediate filament genes (Dunn *et al*, 1998); however, this has yet to be proven. Mice lacking the putative transcription factor MOVO1 also show hair shaft defects, including kinks and intercellular splits (Dai *et al*, 1998). Interestingly, the *Drosophila* homolog of *mOvo1* controls epidermal differentiation, and is transcriptionally repressed by a dTCF/armadillo complex (analogous to LEF/β-catenin) in response to WNT signaling (Payre *et al*, 1999).

Analyses of naturally occurring or induced mouse mutants have identified roles for several other transcription factors in the control of hair shaft differentiation, including a homeobox protein HOXC13 (Godwin and Capecchi, 1998), which may regulate expression of certain keratin-associated protein genes (Tkatchenko *et al*, 2001), and a winged-helix/forkhead transcription factor FOXN1 (formerly WHN) (Nehls *et al*, 1994; Segre *et al*, 1995; Brissette *et al*, 1996), which is mutated in nude mice and regulates expression of an acidic hair keratin gene (Meier *et al*, 1999). A case of two sisters carrying a mutated *FOXN1* gene and displaying hair, nail, and immune defects has been reported, indicating conservation of the role of FOXN1 in mouse and humans (Frank *et al*, 1999). The genes encoding the MSX1 and MSX2 transcription factors are coexpressed in hair follicle matrix cells during anagen, suggesting they may play overlapping roles (Satokata *et al*, 2000). In mice ectopically expressing *Noggin* in the hair matrix, expression of the *HoxC13*, *Foxn1*, *Msx1*, and *Msx2* genes is inhibited, indicating that activation of these genes requires BMP signaling (Kulessa *et al*, 2000). A more detailed analysis of how these various factors interact with each other and with other growth and transcription factors expressed in follicles to produce the normal variety of hair types in humans and other mammals remains a fascinating area for future study.

POLARITY OF THE HAIR FOLLICLE

Hair follicles grow at an angle to the skin, pointing from anterior to posterior, and regulation of this polarity may be controlled in part by *Shh*, which shows an asymmetric pattern of expression in hair and feather follicles (Millar, 1997; Gat *et al*, 1998; Morgan *et al*, 1998). In support of this idea, overexpression of *Shh* in embryonic chick skin causes the formation of enlarged feather buds that have lost their normal orientation (Ting-Berreth and Chuong, 1996a). Perturbations of the WNT signaling pathway, including overexpression of *Wnt7a* or stabilized β-catenin in embryonic chick skin and overexpression of *Lef1* or stabilized β-catenin in transgenic mouse skin result in altered follicular polarity (Zhou *et al*, 1995; Gat *et al*, 1998; Noramly *et al*, 1999; Widelitz *et al*, 1999, 2000), and can induce symmetrical expression of *Shh* in the follicle (Gat *et al*, 1998), suggesting that WNT signals may lie upstream of *Shh* in controlling polarity.

CONTROL OF HAIR FOLLICLE SHAPE

Mutations in the mouse genes encoding TGF-α, the TGF-α receptor (epidermal growth factor receptor), and the transcription factor ETS2, cause altered hair follicle architecture and wavy hair, indicating that these factors play critical roles in governing the shape of hair follicles (Luetteke *et al*, 1993; Mann *et al*, 1993; Luetteke *et al*, 1994; Yamamoto *et al*, 1998). Several other mutations, e.g., *waved coat* (*Wc*) (Kojima *et al*, 2000), cause wavy

THE HAIR GROWTH CYCLE: SIMILARITIES TO MORPHOGENESIS

During postnatal life, hair follicles undergo successive cycles of growth (anagen), regression (catagen), and rest (telogen) (Dry, 1926). Like hair follicle morphogenesis, the initiation of a new growth phase and the subsequent downgrowth, proliferation, and differentiation of the follicle require signaling between follicular dermal and epithelial cells (Oliver and Jahoda, 1988), and recent molecular analyses suggest that signaling pathways active during hair follicle morphogenesis are reutilized in postnatal, cycling hair. At anagen onset *Wnt10b* mRNA localizes to epithelial cells adjacent to the dermal papilla (Reddy *et al*, 2001) and the WNT-responsive TOPGAL reporter gene is activated in the hair follicle bulge, the location of follicle stem cells (DasGupta and Fuchs, 1999). Onset of the first postnatal anagen fails to occur in mice that progressively lose β-catenin from the epidermis and follicular epithelium, indicating that activation of WNT signaling in the epithelium is necessary for this process (Huelsken *et al*, 2001). Isolated dermal papilla cells cultured in the presence of certain WNT proteins maintain their hair follicle-inducing properties, which otherwise are lost after several passages in culture (Kishimoto *et al*, 2000), suggesting that, as in morphogenesis, WNTs play an important part in conveying inductive signals between the follicular epithelium and mesenchyme of postnatal follicles. SHH may also recapitulate some of its morphogenetic functions during anagen. While not required for anagen onset, SHH is necessary for subsequent events of anagen, including proliferation of epithelial cells and downgrowth of the follicle into the dermis (Wang *et al*, 2000), and ectopic expression of *Shh* is capable of inducing resting follicles to enter a growth phase (Sato *et al*, 1999). A plethora of other molecules have been either suggested or proven to play roles in controlling postnatal hair growth cycles, some of which, such as FGF5 and hairless (Cachon-Gonzalez *et al*, 1994; Hebert *et al*, 1994; Ahmad *et al*, 1998), are not required for morphogenesis (reviewed in Cotsarelis and Millar, 2001; Stenn and Paus, 2001).

CLINICAL IMPLICATIONS: WHAT DOES THE FUTURE HOLD?

Knowledge of the molecules and pathways that regulate hair follicle formation and hair growth will be essential for achieving therapeutic goals for hair loss conditions, including the ability: (i) to create new hair follicles; (ii) to change the characteristics (such as size or shape) of existing follicles; and (iii) to alter hair growth in existing follicles, as well as aiding in the search for target antigens important in the etiology of alopecia areata and scarring alopecia (reviewed in Cotsarelis and Millar, 2001). Inhibiting the activities of molecules important for hair follicle formation and cyclical growth may also ultimately provide us with means for treating hirsutism. While we are far from achieving these goals, the identification of molecules such as β-catenin and SHH that are capable of inducing the formation of new hair follicles provides us with potential strategies for treating conditions in which follicles have been completely destroyed. SHH is also able to induce anagen (Sato *et al*, 1999), a property that may be useful for treating a variety of conditions. In designing therapeutic approaches, however, it must be borne in mind that these molecules can also cause the formation of tumors such as pilomatricoma and BCC (Oro and Scott, 1998; Chan *et al*, 1999). It will therefore be important to ensure, by controlling the dose, or modifying the properties of these molecules, that one can induce follicle formation without producing harmful side-effects. The development of methods for delivering genes to hair follicles is an area of active research that will clearly be critical for achieving therapeutic goals (Alexeev *et al*, 2000; Li and Hoffman, 1995; Fan *et al*, 1999; Domashenko *et al*, 2000; Cotsarelis and Millar, 2001).

Although striking advances have been made recently in our

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hair follicles in different regions of the body have different properties, including size, duration of the anagen growth phase, and sensitivity to androgens. The mechanisms by which testosterone impacts on signaling in the hair follicle to achieve its effects are also not understood, an area of importance for the development of novel therapies for androgenetic alopecia. The next few years are likely to bring us answers to some of these outstanding questions, and perhaps the beginnings of therapeutic applications for our recently acquired knowledge.

The author thanks Dr Andrzej Dhigosz for the SHH pathway diagram, Dr George Cotsarelis for comments on the manuscript, Drs Alla Domashenko and George Cotsarelis for samples of human scalp skin, and Dorothy Campbell for histologic preparation. The author is a recipient of a Dermatology Foundation/Dermik Laboratories Career Development Award, and this work was also supported by the National Alopecia Areata Foundation and the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

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