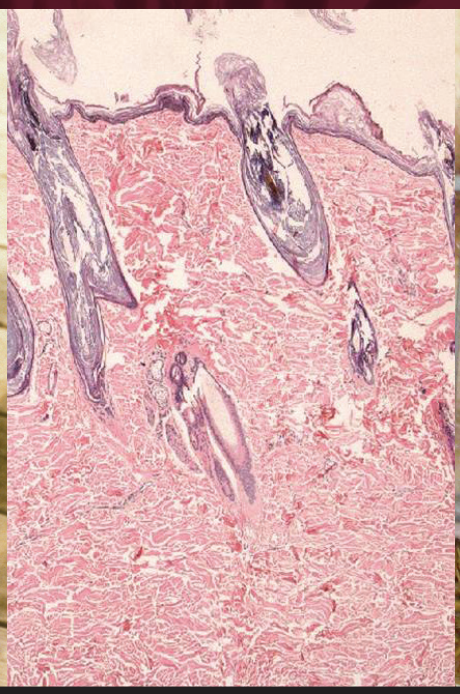
Hair Loss Disorders in Domestic Animals

Lars Mecklenburg, Monika Linek,
and Desmond J. Tobin



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Contents

Foreword

It is with greatest pleasure and enthusiasm that I am writing these introductory lines to what has turned out to be a much-needed, authoritative, and most informative new monograph on hair growth disorders in domestic animals—one of the major topics of clinical dermatology, not only in human but also in veterinary medicine.

The editors have done an admirable job in charting a difficult territory marred by the unavailability of sufficient analytical research, by confusion about key underlying biological principles, by often rather vague pathogenesis concepts, and by contradictory, misleading, or ill-advised terminology. Together with an excellent, international team of expert contributors, the editors are offering solid guidance and a fresh perspective on how to approach alopecias in domestic animals from the vantage point of modern skin and hair biology in a very systematic manner, and thus convincingly fill a void in the available veterinary literature.

As a clinical dermatologist with a special interest in basic hair biology and human hair pathology, I am fully aware of the importance and instructiveness of alopecia in domestic animals for dissecting the principles that control hair follicle cycling, and for studying how cycling disorders can lead to hair loss. I also realize the major, as yet insufficiently exploited potential of domestic animals as excellent models for the study of human hair disease. Therefore, the editors are to be congratulated for calling our attention to the multiple, but often neglected parallels between human and animal hair loss, and for the highly methodical, well-structured approach they are taking in presenting the topic at hand.

Mirroring the often merely historical and commonly descriptive rather than pathobiologically founded classification of human alopecias, the even less studied animal alopecias are notoriously difficult to classify. The editors and chapter authors have done a marvellous job in providing pragmatic guidance and a reasonable working classification that generally avoids to fall into the trap of drawing unadvisedly close parallels to suspected "analogous" human forms of alopecia, whose pathogenesis may well be distinct from what is conceived as a counterpart in domestic animals.

Thus, one does not have to be a prophet to predict that this unique new monograph—a most welcome enrichment of the veterinary dermatology literature—will soon also be consulted by many investigators across a wide range of the life sciences, who are interested in the principles that drive hair loss disorders in mammalian species or who are looking for instructive animal models of specific alopecias, beyond what mice and rats have long offered in this respect.

May this exquisite new book enjoy the exceptionally long "anagen" phase it deserves!

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May 2008

Preface

With the loss of its relevance as a thermal regulator in mammalian survival, the psychosocial significance of hair for humans has continuously increased. Therefore, it is not surprising that domestic animals with alopecia are increasingly presented by their owners to veterinarians for treatment. As with physicians, the veterinary clinician is confronted with a difficult task, since the pathogenesis of alopecia can be very complex and therapeutic possibilities very limited. The aim of this book is to help with the understanding and diagnosis of alopecia and to demonstrate what therapeutic options are available to both the veterinarian and client. As the diagnosis of alopecia frequently relies on a histopathological evaluation of the hair-bearing skin, this book is aimed not solely at practicing veterinarians, but also veterinary pathologists who work with skin biopsies from alopecic patients.

Above all, the diagnosis and treatment of alopecia should be grounded on a thorough understanding of hair follicle anatomy and physiology, and particularly on how this may differ between species. To this end, our book begins with a detailed introduction into the basics of hair follicle biology. Moreover, it provides an insight into the variety of spontaneous alopecic diseases in domestic animals. This will hopefully stimulate new thinking on the etiology and pathogenesis of alopecia, and aid researchers in their search and development of new therapeutics for alopecia.

As Hopwood claimed in 1957, "the urge to classify is a fundamental human instinct; like a predisposition to sin, it accompanies us into the world at birth and stays with us to the end" (Hopwood AT. *Proceedings of the Linnean Society of London* 1957;171:230). As is true for other diseases, the classification of alopecias continues to become more and more complex as new disease entities are described. Our system of classification significantly extends that of previous literature on veterinary alopecia, and reflects this increasing complexity. We consider that histopathologic and clinical features of disease are the most relevant parameters for classification and aim to be as descriptive and precise as possible in our system. This classification however remains in a state of flux, and will no doubt be further improved over the next decades. As was so well expressed by H.E.M. Kay (*The Lancet* 1974;11:586) when addressing lymphoma classification: "This system makes no claim to be comprehensive or even comprehensible, so there may well be scope for other classifications of classifications and ultimately, one hopes a classification of classifications of classifications. At that point we shall need a conference in the Caribbean."

Finally, we would like to thank all those who have enabled us to compile this book. Among many others, these are: Zeineb Alhaidari, Ellen Baumeister, Luc Beco, Kerstin Bergvall, Jan Declercq, Claude Favrot, Raffaella Finocchiaro, Marion Gähle, Szymon Godynicki, Thelma Lee Gross, Renate Hämmerling, Rubert Judd, Miguel Angel Toro Ibáñez, Markus Maget, Kevin McElwee, Claudia Nett, Thierry Olivry, Ralf Paus, Eva M.J. Peters, Sylvia Ruefenacht, Neil Sargison, Rudolf Schwarz, David Steffen, Gudrun Wirth, Pat White, Stephen White, Yulie Yager, and the team from the Section of Clinical Dermatology and the Institute of Veterinary Pathology of the Vetsuisse Faculty, University of Berne.

Lars Mecklenburg
Monika Linek
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May 2008

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Part 1

Hair Follicle Biology

1.1 Ontogeny of the hair follicle

Desmond J. Tobin

Both humans and domestic animals communicate significantly via their physical appearance, and the hair fiber-producing mini organ called the hair follicle accounts for much of the variation in domestic mammal phenotype. Although commonly dismissed as being of superficial importance, the hair follicle is truly one of the nature's most fascinating structures (Chuong 1998). Hair growth, one of the only two uniquely mammalian traits (the other is the mammary gland), serves several important functions. These include thermal insulation, camouflage, social and sexual communication, sensory perception, and protection against trauma, noxious insults, insects, and so on. These features have clearly facilitated evolutionary success in animals.

The hair follicle or, as it is known in humans, the "pilosebaceous unit" encapsulates all the important physiologic processes found in mammalia, including controlled cell growth and death, interactions between cells of different histologic type, cell differentiation and migration, and hormone responsitivity. Thus, the value of the hair follicle as a model for biological scientific research goes way beyond its scope for cutaneous biology or dermatology alone. Indeed, the recent and dramatic upturn in interest in hair follicle biology has focused principally on the pursuit of two of biology's holy grails: post-embryonic morphogenesis and control of cyclical tissue activity.

If one first considers the role of the skin, arguably our body's largest organ, as the mammal's sensor at the periphery (a veritable "brain on the outside"), one can begin to appreciate some of the contributions its principal appendage, the hair follicle, can make. The skin incorporates all major support systems found in the body: blood, muscle, and innervation as well as its role in immunocompetence, psycho-emotion, ultraviolet radiation sensing, and endocrine function, among others. These participate in the homeostasis of the mammalian body. Not surprisingly, therefore, the skin contains several reservoirs of stem cells located in the epidermis, the hair follicle, and perhaps also the sebaceous gland.

The hair follicle is formed from a bewilderingly complex set of interactions involving ectodermal, mesodermal, and neuroectodermal components, which go to elaborate five or six concentric cylinders in humans of at least 15 distinct interacting cell subpopulations. These together provide a truly exceptional tissue that rivals the vertebrate limb bud as a model for studies of the genetic regulation of development. Much of the research on the regulation of hair follicle development or morphogenesis has been carried out in murine models, especially the mouse (Schmidt-Ullrich and Paus 2005). While some species-specific (and even intraspecies) differences are expected (Drögemüller et al. 2007), it is considered likely that broadly similar pathways and molecular regulators will operate in all mammals. Thus, the discussion below will largely refer to data that continues to emerge from studies in mice, given the ready availability of powerful mouse genetics.

The mysteries of the "creation" of the hair follicle in mammalian skin have only just recently begun to be unraveled. As with any highly complex multicellular structure that has experienced enormous evolutionary selective pressure, nature's master builder has designed the hair follicle with multiple levels of redundancy: with backup systems and with backup systems for these backup systems. A plethora of excellent reviews have appeared over the last 10 years that describe the exquisitely complex molecular mechanisms active during hair follicle development (Fuchs

et al. 2001; Millar 2002; Botchkarev and Paus 2003; Schmidt-Ullrich and Paus 2005). It is attempted here to provide a comprehensive overview of some of the latest developments in this fascinating process. This is a rapidly developing field however, and new molecular regulators are being added to these pathways continually.

1.1.1 Skin development

The development of the forerunner of skin epithelium, the neuroectoderm, occurs after gastrulation in the embryo. Whether this neuroectodermal tissue develops into skin epithelium or continues to develop as neuronal tissue is determined by signaling via the Wnt pathway, which renders some of the ectoderm nonresponsive to fibroblast growth factors (FGFs). In the absence of FGFs the cells of the developing ectoderm can instead respond to another class of morphogens called bone morphogenetic proteins (BMPs), which help direct the ectodermal cells to form a layer of multipotential skin epithelium (cf. Botchkarev and Sharov 2004). The Wnt gene (named from the *Drosophila* gene "Wingless" and its vertebrate homologue "Int") was first identified in *Drosophila melanogaster* to function in embryogenesis and adult limb formation. Wnt is now known to represent a major class of secreted morphogens that are critical for the establishment of pattern development in all multicellular organisms.

The skin epithelium lies above a dermatome-derived mesenchymal or embryonic connective tissue primarily consisting of collagen-producing fibroblasts. Here, too, Wnt signaling determines the fate of dermis fibroblasts (Atit et al. 2006). A third major component of the developing skin is the neural crest-derived melanocyte, a cell that produces melanins. Primitive melanocytes (sometimes referred to as melanoblasts) migrate from the neural crest, through the dermis, and populate the basal layer of the epidermis.

Hair follicle formation in the developing skin requires communication between specialized mesenchymal cells of the dermis and epithelial cells of the epithelium above. This interaction is very complex and operates via pathways that direct specialized outcomes for both the epithelial (keratinocytes) and dermal cells (fibroblasts). Much debate in the literature remains centered on the hierarchy of the increasing numbers of factors implicated in hair follicle morphogenesis, and on the degree of their redundancy. Of particular interest has been the nature of the first signals that determine where along the overlying epidermis a hair follicle is likely to be produced (so-called 'patterning').

As an aid to conceptualizing the impact of these molecular signaling events, it may be useful to associate them directly with morphologically distinct stages in hair follicle development, as significant morphologic change is required to form a fully developed hair follicle. These changes can be broadly separated into the following phases: (a) induction/initiation, (b) organ downgrowth, and (c) cellular differentiation. A useful guide to aid recognition of these distinct stages has been prepared for C57Bl6 mice (Paus et al. 1999). Therein, the preplacode stage of hair follicle development is assigned as Stage 0, while the clustering of keratinocytes in the basal layer of the epidermis that lengthen and assume an altered "north–south" polarity to form the hair follicle placode stage is referred to as Stage 1 (Fig. 1.1.1).

1.1.2 Hair follicle placode formation

It is now considered most likely that the first signal(s) that trigger(s) the formation of the regularly spaced epithelial thickenings called hair follicle "placodes" emerge(s) from the mesenchyme (Olivera-Martinez et al. 2004). This is derived from the evidence of rather uniform β-catenin accumulation in dermal cells under the control of Wnt, effective only at short distances. Subsequently, β-catenin translocates to the nuclei of some of these dermal cells, where it can form complex with lymphoid enhancer factor (LEF) family members for DNA binding and subsequent gene activation (Noramly et al. 1999). It is of note that while LEF expression is required in the mesenchyme before initiation of vibrissae development is possible (Kratochwil et al. 1996), loss of LEF in pelage skin results in only a partial reduction in hair follicle development overall, suggesting the existence of hair follicle type-specific controls and also a degree of functional redundancy. In a similar way it has been suggested that BMP-associated inhibition may operate in several different ways, to specify the development of hair follicles of different types, and thereafter may additionally affect hair follicle density (Fuchs 2007). The BMPs, a subfamily with over 16 distinct secreted signaling protein members, belong to the transforming growth factor beta (TGF-β) superfamily of proteins.

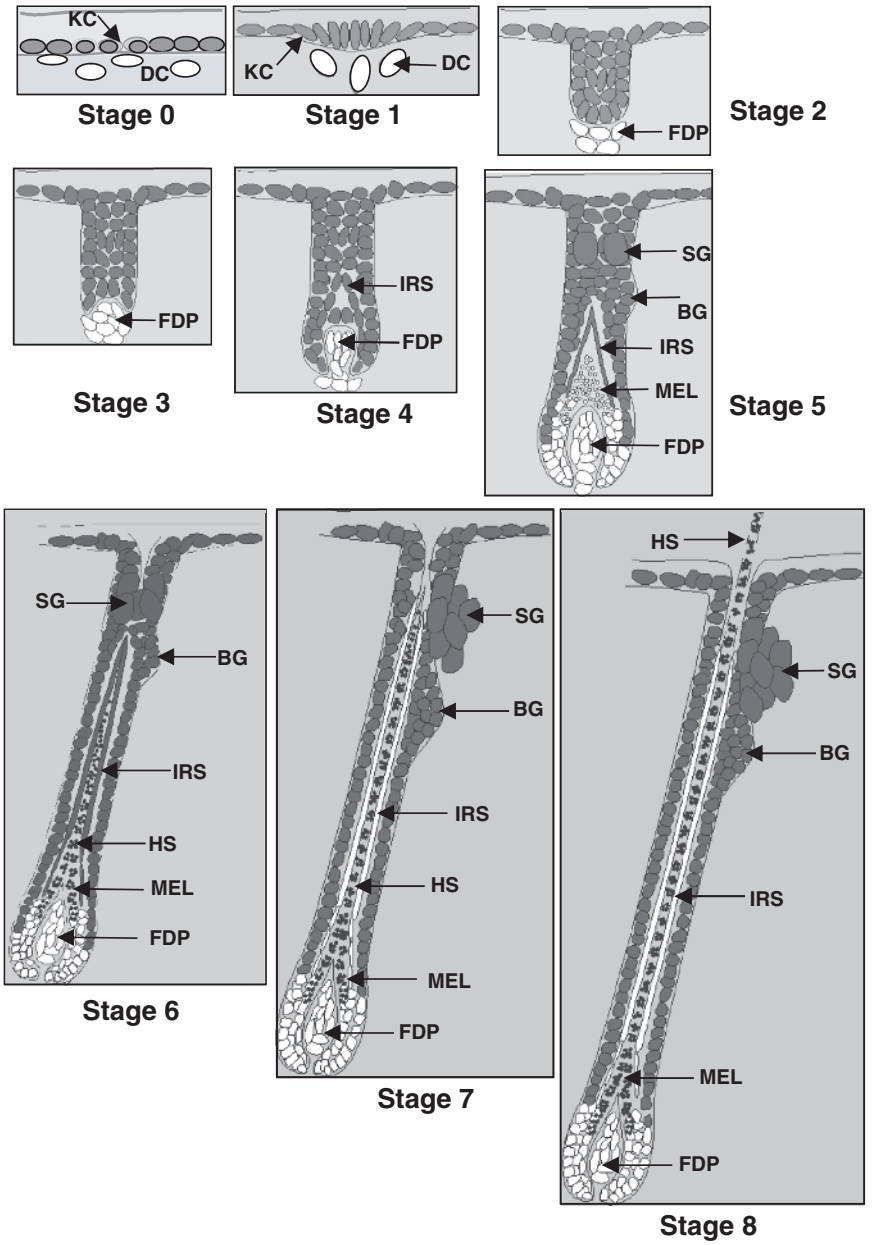

Fig. 1.1.1 Cartoon of hair follicle morphogenesis. Stages 0–8 according to Paus et al. 1999. KC, keratinocytes; FDP, follicular dermal papilla; DC, dermal mesenchymal cells; SG, sebaceous gland; BG, bulge; IRS, inner root sheath; HS, hair shaft; MEL, melanin. (Modified from Tobin (2005).)

Readers should also be aware that for any given species, and even within the same species, the skin can produce hair follicles of several different types that generate different types of hair fiber (Maderson 2003). The molecular regulation underlying this diversity is of significant interest. There is evidence in the mouse that the control of placode initiation to form any of the four different types of hair fiber involves different regulatory controls. For example, ectodysplasin signaling is required for guard hair development, while the development of secondary hairs (i.e., "awl" and "auchene" hairs in mice) involves ectodysplasin-independent

signaling. The latter, however, is instead dependent on the Wnt/β-catenin and Noggin/LEF pathway (Schmidt-Ullrich and Paus 2005). NFκB signaling is required in early hair follicle induction downstream of ectodysplasin (Schmidt-Ullrich et al. 2001). NFκB transmits the ectodysplasin signal that activates sonic hedgehog (Shh) and cyclin D1 expression, responsible for postinitiation hair placode downgrowth (Schmidt-Ullrich et al. 2006).

As a consequence of the initial dermal signal, the epithelium is induced to initiate the expression of molecules that act as either promoters or repressors of placode formation. Molecular participants in this initial signaling to Wnt-responsive epithelial cells in mice include the FGFs (e.g., FGF10, FGF7), inhibitors of BMPs (e.g., noggin), and inhibitors of Wnt (e.g., Dickkopf) (Jung et al. 1998; Botchkarev et al. 1999; Sick et al. 2006). Indeed, hair follicle density is reduced where there is either an excess BMP production, a failure to inhibit BMP (e.g., via an absence of noggin), or via lack of FGF receptor (Petiot et al. 2003; Mou et al. 2006). Thus, hair follicle formation at a particular site in the epidermis results from a highly orchestrated interaction of mesenchyme with epithelium. This process responds to dynamic reaction–diffusion type gradients of stimulating/inhibiting signals generated from secreted factors and their cognate receptors. Also implicated in this complex process are transcription factors (proteins that work with other proteins to either promote or suppress the transcription of genes) and cell adhesion molecules.

The activation of the Wnt signaling pathway is an absolute requirement for the initiation of Stage 1 of hair follicle morphogenesis. Currently 19 distinct members of the Wnt family of ligands (and over 10 distinct receptors, named "frizzled") have been identified in humans and mice, and it is not yet clear which of these are involved in Stage 1 initiation. It has recently been shown that Wnt10b promotes the development of hair follicles in a mouse embryonic skin tissue model (Ouji et al. 2006). While Wnt10b is uniformly expressed in embryonic mouse skin, this molecule becomes strikingly upregulated in the hair follicle placode (St-Jacques et al. 1998; Reddy et al. 2001). Indeed, the expression of Wnt10b is abolished in ectodysplasin-mutant mice that exhibit a hypoplastic hair formation. Moreover, a mutation in the gene for the ectodysplasin receptor (EDAR) (named "downless" in mice) results in a similar phenotype (Headon and Overbeek 1999). Consequently, in the absence of EDAR, neither Shh nor BMP4 is expressed (Barsh 1999).

It is important to note that the Wnt pathway is active in both the epithelial and mesenchymal components of developing hair follicles (DasGupta and Fuchs 1999). Activation of the Wnt signaling pathway is sufficient for induction of hair follicle development. Similarly, if β-catenin (i.e., the mediator of Wnt signals) is lost from murine epidermis, no hair follicles will develop (Huelsken et al. 2001), and if β-catenin signaling is excessively stimulated, excess hair follicles will develop (Gat et al. 1998; Lo Celso et al. 2004). Moreover, BMP (i.e., BMP2, −4, and −7) as well as Shh expression (but not ectodysplasin) are dependent on β-catenin, suggesting that expression of ectodysplasin lies upstream of Wnt activation in the epithelium (Huelsken et al. 2001).

The reaction and diffusion model for biological patterning proposed originally by Alan Turning in the 1950s and recently demonstrated experimentally for Wnt and its antagonist "Dickkopf" in murine skin (Sick et al. 2006) permits an understanding of hair formation based on the control of these molecules by positive and negative regulators expressed either in the developing hair follicle itself or in the surrounding tissues. Examples of negative regulators for hair follicle development include the BMPs and activinαA. These inhibitors can in turn be inhibited by noggin and follistatin, respectively. Mice lacking noggin have slower hair follicle development and also fewer total hair follicles formed (Botchkarev et al. 1999). Moreover, these mice also exhibit a reduced expression of LEF and this may suggest that its expression is also controlled by BMP. BMPs appear to inhibit hair follicle initiation. Several other secreted molecules are expressed in developing hair follicles where they can inhibit BMP activity. These include follistatin, an inhibitor of activinαA. TGF-β2 (which belongs to the same family as activinαA) is expressed in the placode epithelium and, when administered to mesenchyme in the absence of overlying epithelium, can induce the formation of follicular papilla (Foitzik et al. 1999). In contrast, mice lacking the TGF-β2 gene exhibit retarded hair follicle morphogenesis and have fewer hair follicles.

Several other potential players that can either stimulate or inhibit hair follicle development have been suggested from recent research including the

transcription factors Msx1 and Msx2 (Satokata et al. 2000); signaling via the epidermal growth factor receptor (Kashiwagi et al. 1997); extracellular matrix proteins including laminin-10 (Li et al. 2003); and neurotrophins and their receptors (Botchkarev and Paus 2003). Recently, a role for the chromatin remodeler Mi-2beta in hair follicle morphogenesis has been reported (Kashiwagi et al. 2007). This study showed that depletion of Mi-2beta blocked the induction of hair follicles in mice. While Mi-2beta appears to be dispensable for the maintenance of established repopulating epidermal stem cells, this transcription factor appears to be essential for reprogramming basal cells of the developing epidermis into follicular and, subsequently, hair matrix fates (Kashiwagi et al. 2007).

Exciting new research has demonstrated that hair follicle neogenesis may occur in adult wounded skin, if the wound site is of sufficient size and the wound is allowed to repair physiologically (Ito et al. 2007). This example of reported neofolliculogenesis occurred via an upregulation of Wnt expression, and was shown experimentally by either inducing high Wnt expression or by inhibiting its expression to reduce the hair follicle number (Ito et al. 2007). This study may be the first formal demonstration that hair follicle numbers may not be preset at the embryogenesis state but can be increased as a function of wound healing in the adult animal. There may be room, however, for contribution from stem cells from hair follicles located at the edge of the wound after migration into the wound center.

1.1.3 Formation of the hair follicle mesenchyme

While formation of the hair follicle placode is being initiated, increasing organization of dermal cells underlying this epithelium structure becomes evident. A signal from the placode is thought to determine and direct the clustering of the dermal fibroblasts to form the "dermal papilla." The dermal condensate fails to develop in the absence of β-catenin expression (Huelsken et al. 2001), indicating a crucial role for Wnt signaling in the development of the early dermal papilla (DasGupta and Fuchs 1999). Moreover, efficient development of the dermal papilla appears to be influenced by cross talk between the epidermal placode and the developing dermal papilla via signaling through the platelet-derived growth factor A (PDGF-A) expressed in the epithelial placode and its cognate receptor PDGF-R in the dermal condensate (Karlsson et al. 1999). This is suggested by the observation that mice deficient in PDGF generate hypoplastic hair fibers, characterized by dermal papilla of reduced size. Presumably these cells reduce secretion of hair growth inducing morphogens and mitogens. Recent work suggests that BMP6 may have a dermal papilla-associated regulatory control in the hair follicle (Rendl et al. 2008).

Another early gene involved in the organizing of the mesenchyme to form a dermal papilla is Shh (St-Jacques et al. 1998; Oro and Higgins 2003; Levy et al. 2005). Mice lacking Shh still exhibit placode initiation and formation of the dermal condensate (Iseki et al. 1996). Although these data indicate that Shh signaling lies downstream of Wnt/β-catenin signaling, Wnt5a is expressed in the developing dermal condensate of wild-type but not Shh$-/-$ embryos, suggesting that this Wnt is a target of Shh in hair follicle morphogenesis. Thus, while Shh is not critical for the first epithelial signal, nor perhaps even for the initial gathering of the presumptive dermal papilla fibroblasts, it is required for subsequent hair follicle downgrowth and for subsequent papilla formation. Evidence for the latter can be gleaned from the coexpression in the forming papilla of Shh pathway-related molecules including Patched1 (i.e., the Shh receptor) and Gli1 (i.e., a Shh transcription factor) (Oro and Higgins 2003). Recent data from Gli1 knockout mice, however, suggest that some hair types can still develop in the absence of Gli1 (Mill et al. 2003). This finding suggests that different hair types have different requirements for Gli1 signaling. By contrast, the loss of smoothened (an obligate component of all Shh signaling) results in severely affected hair follicle morphogenesis (Gritli-Linde et al. 2007). The increasing inductive capacity of the developing dermal papilla may be indicated by upregulation of hepatocyte growth factor and versican at this stage (Kaplan and Holbrook 1994).

1.1.4 Hair follicle downgrowth

"Second" signals from the developing dermal papilla induce proliferation in the follicular epithelium to drive the initial downgrowth of the epidermal placode to form the Stage 2 hair follicle. These initial hair follicle "commitment" events presage subsequent

active epithelial–mesenchymal cross talk that drives cytodifferentiation to determine which cells will go on to form keratinocytes of the hair fiber and follicular sheaths, and which fibroblasts form the growth-inducing follicular papilla versus the dermal sheaths. The cell fate-determining "signals" are mediated by intercellular signaling molecules secreted from specific subpopulations of skin cells and that have multiple receptors/targets to transduce their effects.

Of note here is the exquisite specificity of the signaling events occurring between different cell subpopulations. For example, recombination experiments have shown that dermis taken from one body region when combined with epidermis from another body site (even in another individual) will direct the formation of hair follicles that are characteristic of the dermis donor site. A test of this in humans was recently carried out whereby male follicular dermal tissue taken from the scalp directed the formation of terminal hairs when recombined with female arm epidermis (Reynolds et al. 1999).

One of the enigmas of hair follicle development is how a relatively undifferentiated cluster of epithelial and mesenchymal cells can give rise to such a large number of distinct cell lineages with variable different differentiated products (Fig. 1.1.2). It is clear from knockout mice that downgrowth will only occur in the presence of Shh (Chiang et al. 1999). Although Shh engages in highly significant signaling events between hair follicle epithelium and mesenchyme, the identification of the pathway activated by the Shh signal in this process remains unknown. ActivinαA and hepatocyte growth factor (HGF) may be possible candidates. ActivinαA not only is a secreted signaling molecule expressed by the early dermal papilla cells, but is also inhibited by follistatin (also a BMP inhibitor). Consequently, mice lacking either activinαA or follistatin produce defective vibrissa follicles (Millar 2002). On the other hand, mice that overexpress HGF not only exhibit accelerated hair follicle development, but also produce more hair follicles (Lindner et al. 2000). It is also likely that adhesion molecules and extracellular proteins are also important contributors to these developmental processes.

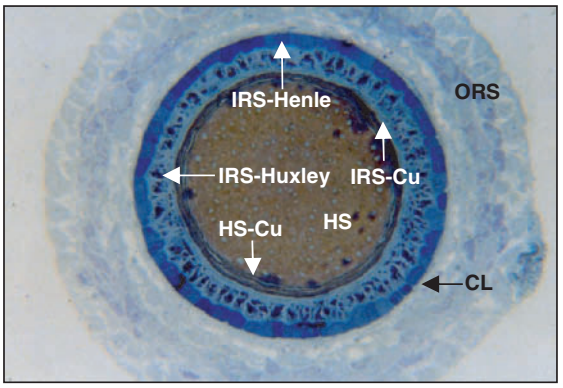

Fig. 1.1.2 High-resolution light microscopy image of a transverse section of a human hair follicle cut at the level where maximum cell lineage commitment occurs (suprabulbar level). IRS, inner root sheath; ORS, outer root sheath; HS, hair shaft; Cu, cuticle; CL, companion layer.

Hair follicle morphogenesis (Fig. 1.1.3) is morphologically appreciable only at Stage 3 when the forerunner of a hair follicle "bulb" becomes evident—a process reflecting the activation/induction of multiple keratinocyte differentiation pathways that lead to considerable structural change within the tissue (Fig. 1.1.3d). At Stage 3 the developing hair follicle appears as a "peg" of tissue consisting of an elongated column of concentrically layered keratinocytes—at least seven different layers of epithelium can be appreciated at this stage. A little later at Stage 4 (Fig. 1.1.3e) the mesenchymal component of the hair follicle, that is, the follicular dermal papilla, is now located within the cavity of the developing epithelial bulb. The hair peg continues to elongate into the dermis of the skin during this stage and there is evidence now of the formation of the inner root sheath (IRS), as it assumes a cone-shaped structure. The follicular papilla becomes progressively invaginated by the enlarging epithelial hair bulb.

1.1.5 Formation of the hair follicle inner root sheath

The emergence of such an impressive differentiation programming in the hair follicle epithelium at Stages 3–5 (Fig. 1.1.3d–f) is of course dependent on the spatiotemporal expression of relevant cell fate genes. Moreover, it is during this stage of hair follicle development that the additional processes that see the hair follicle's polarity and shape occur (see below). One of the first and most striking events of cytodifferentiation within the developing hair follicle is the emergence of the IRS layer of the hair follicle wall during late

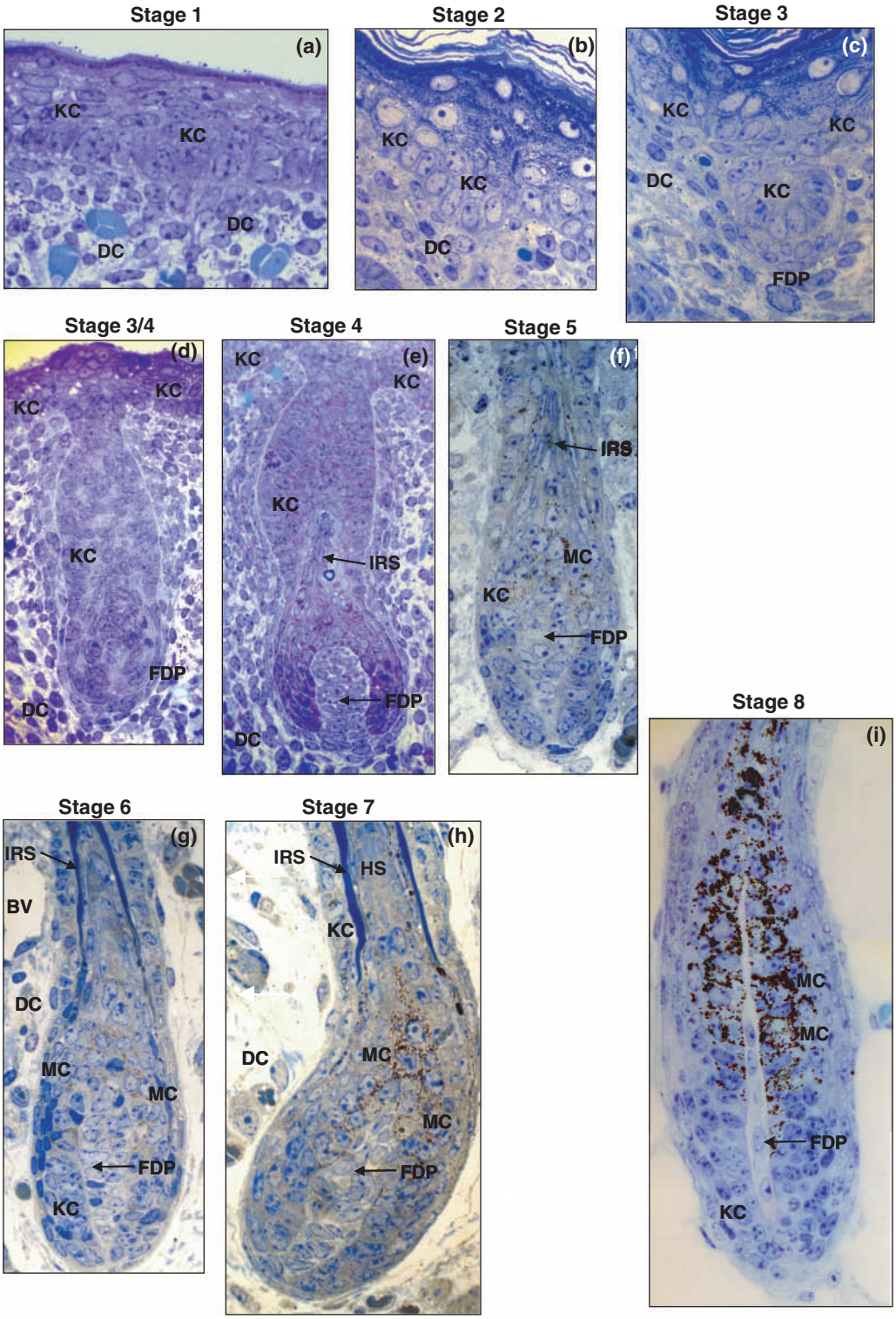

Fig. 1.1.3 High resolution light microscopy images of hair follicle development in mouse skin. Stages 0–8 according to Paus et al. 1999. KC, keratinocytes; FDP, follicular dermal papilla; DC, dermal mesenchymal cells; IRS, inner root sheath; HS, hair shaft; MC, melanocytes; BV, blood vessel. (Modified from Tobin (2005).)

Stage 3. Signaling via the BMP receptor is also important for the determination of matrix keratinocyte differentiation into both IRS and hair shaft keratinocytes (Ming Kwan et al. 2004). Indeed, if the BMP receptor R1A is lacking, matrix keratinocytes fail to differentiate and instead matrix cell tumors occur.

The cells of the hair bulb matrix that go on to develop into IRS and hair shaft begin to express genes encoding for Notch1. At this stage this membrane-associated receptor protein interacts with its ligands Jagged-1 and Jagged-2 (Favier et al. 2000), which is then cleaved by secretase to yield a nuclear intracellular cofactor. Interestingly, in hair follicles deficient in secretase, IRS cells fail to retain their IRS fate and the affected hair follicles appear to undergo a reversion to epidermal differentiation fate with resultant cyst formation (Blanpain et al. 2006). Transgenic mice expressing a constitutive activated form of Notch1 under the control of the involucrin promoter were reported to develop both skin and hair abnormalities. Here, hair follicle IRS differentiation is significantly delayed, resulting in a "Mohawk"-like alopecia associated with the anagen phase of the hair cycle (Uyttendaele et al. 2004). Furthermore, when overexpression of Notch1 was directed to precortical keratinocytes, the differentiation of hair shaft medullary cells was disrupted and yielded a wavy hair phenotype (Lin et al. 2000).

Cells that differentiate into IRS keratinocytes begin to express components of the cornified envelope (e.g., loricrin, involucrin, and transglutaminases) that make them similar to suprabasal keratinocytes. However, they also begin to express trichohyalin protein, a cross-linker of keratin filaments, in this layer. Trichohyalin is also found in cells forming the medulla (Alibardi 2004). Signaling via the epidermal growth factor receptor (EGFR) can cause defects in the keratinization of the IRS, loss of cohesion with the hair shaft within, and may also result in the production of wavy hair fibers (Luetteke et al. 1993; Hansen et al. 1997).

Another control of IRS cell fate is the transcriptional regulator "CCAAT displacement protein" (CDP, Cutl1), that is, a transcription factor involved in the regulation of cell growth and differentiation. Mice with a null mutation in Cutl1 develop an abnormal pelage due to disrupted hair follicle morphogenesis with reduced IRS formation. The recent observation of Cutl1 expression in cells of the so-called companion layer suggests that this mysterious layer is really the fourth and most external layer of the IRS (Ellis et al. 2001; Gu and Coulombe 2007). The transcription of Shh- and IRS-specific genes is deregulated in Cutl1 mutant hair follicles, suggesting that the progenitors and cell lineages of the IRS specifically express Cutl1 (Ellis et al. 2001). By contrast, it appears that progenitors of the nonkeratinizing outer root sheath are not derived from the differentiating matrix keratinocytes (as in the IRS and hair shaft lineages), but rather may derive from laterally migrating cells from the bulge (Panteleyev et al. 2001).

The expression of the winged-helix/forkhead transcription factor FOXN1 in the IRS and hair shaft is thought to function in the differentiation of these cell types (Lee et al. 1999). Moreover, mice overexpressing FOXN1 in the IRS exhibit disruptions of hair shaft formation (Prowse et al. 1999), whereas a spontaneous mutation in FOXN1 is associated with hair, nail, and immune defects (Frank et al. 1999; Mecklenburg et al. 2005).

1.1.6 Formation of the hair shaft

One of the main ways to distinguish each of the different cell layers of the developing hair follicle is by analyzing the expression of particular keratin genes (Schweizer et al. 2007). Indeed, some of these keratin genes are reported targets of the Wnt gene (Merrill et al. 2001). For example, Wnt/β-catenin/LEF signaling is considered to be very important for differentiation of matrix keratinocytes into precortical keratinocytes. Not only do the promoters of some hair keratin genes contain LEF binding sites and precortical keratinocytes express LEF, but also hair follicle development ceases before the formation of the hair shaft (i.e., Stage 5/6) (Fig. 1.1.3f and g) in LEF knockout mice (DasGupta and Fuchs 1999). This indicates that this stage of hair follicle development is in part regulated by Wnt signaling. As mentioned above, transcription regulators of hair shaft genes also include FOXN1 and HOXC13, which are expressed in hair shaft differentiation cell lineages. Mutating HOXC13 can result in brittle hair alopecia (Godwin and Capecchi 1998), while FOXN1 is mutated in the nude mouse (Mecklenburg et al. 2005) where it results in alopecia and in the absence of keratin K33 in truncal hair follicles (Meier et al. 1999).

The interrelated nature of signaling pathways in hair follicle development is further evidenced by the

finding that the expression of both HOXC13 and FOXN1 can be regulated by BMP signaling (Kulessa et al. 2000). Cells in the hair bulb matrix that are destined to become hair shaft cortical keratinocytes also express BMP4. The expression of Noggin (i.e., the inhibitor of BMP4) can disrupt the differentiation of the cortical keratinocytes needed for hair shaft formation.

It has long been suspected that the IRS plays a significant role in modulating the phenotype of the initially pliant hair shaft within the lower hair follicle. Thus, defects in IRS development (e.g., caused by mutations in Gata-3) are likely to result in the impairment of the hair fiber phenotype (Kaufman et al. 2003). The conditional knockout of the Gata-3 gene from the hair follicle results in mice with a range of phenotypes. These include abnormal and delayed postnatal growth and development, aberrant hair follicle tissue organization, and irregular hair pigmentation. When the transcriptome of these mice was analyzed, the affected hair follicles exhibited upregulation of Notch, Wnt, and BMP signaling pathways (Kurek et al. 2007). Similarly, mice with a conditional knockout of the transcription factor gene Runx1 also display a hair shaft structural deformation in zigzag hairs (Raveh et al. 2006).

Finally, a role for the transmembrane desmosomal cadherin, desmoglein 4, in the development of the hair shaft has been suggested recently. This is supported by observations that mutations in this gene can cause hypotrichosis in humans, mice, and rats. In human hair follicles desmoglein 4 is expressed specifically in the hair cortex and in the cuticle of the IRS and hair shaft, suggesting that this molecule is very important for trichocyte adhesion of the hair shaft (Bazzi et al. 2006).

The events underlying Stage 0 to Stage 5 of hair follicle morphogenesis (Fig. 1.1.3a–f) is a rapidly growing field, and it is likely that we are only at the beginning of elucidating the full range of molecular players involved in hair follicle differentiation.

Significant additional morphologic features are apparent by Stage 5 of hair follicle morphogenesis (Fig. 1.1.3f). Not only does the IRS continue to develop and extend upward within the hair follicle, but several epithelial prominences or bulges also appear along the external wall of the developing hair follicle or outer root sheath (Fig. 1.1.1). One of these "bulges" will generate the future repository of the hair follicle stem cells (e.g., for both epithelial cells and melanocytes). A second more distal bulge will become a site of specialized lipid-forming epithelial cells that will form the holocrine sebaceous gland. Sebum flow from this gland coats and lubricates the hair shaft surface, and may also have antimicrobial function. Differentiation of immature keratinocytes to sebocytes is thought to be regulated in part by the product of the proto-oncogene c-Myc, as this occurs independently of the other cell lineages of the hair follicle. It is thought that c-Myc stimulates some epithelial cells to leave the stem cell compartment and differentiate into sebocytes rather than hair follicle lineages (Arnold and Watt 2001). The transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) is a second transcription factor that specifically regulates the differentiation of sebocytes (Rosen et al. 1999).

1.1.7 Development of the hair follicle pigmentary unit

The developing hair follicle contains—in addition to epithelial keratinocytes and mesenchymal fibroblasts—cells of neuroectodermal origin, the melanocytes. Cutaneous melanocytes of both the epidermis and pilosebaceous unit originate from pluripotent cells that commit to the melanocyte lineage while in the embryonic neural crest. To reach the skin, so-called melanoblasts leave the neural tube and migrate dorsolaterally and differentiate along stereotypical routes from the closing neural tube, migrate between the dermamyotome of the somites and the overlying ectoderm, until they enter the dermis (Rawles 1947). Melanoblasts migrate under the control of specific growth factors (e.g., endothelin-1, stem cell factor/c-kit) through the dermis and subsequently to the epidermis, and distribute within the developing hair follicle. Disruption of the stem cell factor/c-Kit signaling pathway interferes with not only the survival and migration of these early melanocytes, but also their differentiation during this stage of hair follicle morphogenesis.

Much of our knowledge of the events involved in the development of melanocyte compartments within the skin and hair follicle derives from the analysis of gene mutations in mice that affect differentiation, proliferation, and migration of melanocyte precursors (Jackson 1994). There are over 100 genes shown so far to affect hair color in the mouse (Nakamura et al. 2002), and equivalents of many of these continue to be

described in other mammals, where mutations range from total loss of all-over pigmentation (e.g., types of albinism) to loss of pigment from specific body sites (e.g., piebaldism) (Fleischman et al. 1996). Recently, a role for the Notch signaling pathway in the maintenance of melanoblasts and melanocyte stem cells was proposed, where severe coat color dilution resulted when the Notch pathway was disrupted. Loss of hair pigment was also seen when Notch1 and/or Notch2 receptors were ablated in melanocytes (Schouwey et al. 2007).

Even before the onset of hair bulb melanogenesis, that is, around morphogenesis Stage 4 (Fig. 1.1.3f and g), strongly c-Kit and S100 positive cells are visible in selected cells of the hair follicle placode and plug. Later these cells become increasingly dendritic and invade the hair bulb (Peters et al. 2002). There, they appear to distribute as either "dihydroxy-phenylalanine (DOPA)-positive" or "DOPA-negative" melanocytes (Chase et al. 1951), where DOPA positivity refers to their ability to produce the rate-limiting enzyme for melanin formation called tyrosinase or whether it can oxidize DOPA and produce the melanin precursor dopaquinone. As hair follicle morphogenesis progresses to the stage of synthesizing a hair fiber, DOPA-positive melanocytes cluster around the apex of the dermal papilla in the hair bulb. Other amelanotic DOPA-negative melanocytes occupy positions in the outer root sheath (Fig. 1.1.1). The first melanin granules are evident in precortical keratinocytes at this stage.

1.1.8 Final stages of hair follicle morphogenesis

The forming fiber now starts to pass through the hair follicle core in order to exit the skin surface. Its passage through intact follicular epithelium is facilitated by the formation of a "hair canal" (Robins and Breathnach 1970), which is constructed via focal cell death or apoptosis. The developing hair follicle continues to extend deeper and deeper into the skin until its proximal bulbar end is situated within the adipocyte-rich subcutis. Anatomic features characteristic of Stage 6 hair follicles include an increasing complexity of the now multilayered IRS (Fig. 1.1.3e–h). Furthermore, the hair shaft can now be visualized within the hair canal and melanin granules can be seen within its cortical keratinocytes. The final two stages of hair follicle development are characterized by the growth of the hair shaft through the IRS and hair canal until the tip of the fiber emerges from the surface of the skin (Figs. 1.1.1 and 1.1.3h). In addition, the aspect of the sebaceous gland changes relative to the hair follicle horizontal axis at this stage. Finally, the hair follicle attains its maximal length and bulk during Stage 8 when the distal hair shaft is positioned well, free of the skin surface (Figs. 1.1.1 and 1.1.3i).

The anatomy of the fully developed Stage 8 hair follicle is very similar to the anatomy of the growing/cycling anagen hair follicle in the adult (see Section 1.2.1). Epithelial and mesenchymal cells of the developing hair follicle therefore contrive to produce the mature hair follicle via massive cell proliferation and cell differentiation. However, considerable tissue sculpting is also required. Such sculpting events also require intermittent and highly localized programmed cell death (Magerl et al. 2001). Only in this way can this hair follicle assume its full hair shaft-forming status.

Cessation of hair fiber production (i.e., hair shaft growth) during Stage 8 of hair follicle development signals entry into the "hair growth cycle," from which the hair follicle usually does not escape during the life of the individual (see Section 1.2.1).

In summary, the development or morphogenesis of the hair follicle provides an exquisite example and model system to study the earliest communications involved in organ development. It is likely that this system will reveal much about how cells communicate to aid the development of treatments for developmental abnormalities in general and those affecting the hair follicle (e.g., congenital hyper/hypo/a/-trichosis) in particular.

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1.2

Anatomy and physiology of the hair follicle

Desmond J. Tobin

The hair follicle is formed from a bewilderingly complex set of interactions involving ectodermal, mesodermal, and neuroectodermal components, which go to elaborate five or six concentric cellular cylinders of at least 15 distinct interacting cell subpopulations (Philpott and Paus 1998; Botchkarev and Paus 2003).

Much, if not most, of current hair research is conducted in murine models (after an extended period of time when this was dominated by research on wool for economic reasons), and particularly in black-haired mice strains like C57BL6 (Paus et al. 1990). While it is likely that there will be some important species-specific differences, the consensus is that the fundamentals of hair growth will be broadly similar in all mammals. Unlike many other mammals, humans have all but lost their ability to grow hair synchronously (i.e., as a wave). Instead, human hair grows in a mosaic pattern where significant autonomy for growth and pigmentation resides in individual hair follicle.

1.2.1 The hair growth cycle

As discussed earlier (see Section 1.1.8), the fully developed Stage 8 hair follicles actively produce hair fibers. Each follicle will continue to do so until it is induced to enter the first hair growth cycle. Thus, hair cycling paradoxically begins with a regression phase characterized by a massive cell death via apoptosis to yield the first catagen. Upon completion of this catagen phase, the hair follicle will have lost up to 70% of its original Stage 8 tissue mass, as it enters a period of relative rest during telogen. After some time the telogen follicle is reactivated to enter into the first anagen phase of the hair cycle. Thereafter, the hair follicle continues with lifelong cyclical activity (Fig. 1.2.1) until death of the individual (Fuchs et al. 2001; Stenn and Paus 2001).

1.2.1.1 First anagen

This first anagen of the hair growth cycle morphologically resembles several aspects of hair follicle development in utero. In this way the lifelong cyclical activity of the hair follicle recapitulates, at least in part, several embryologic events involved in hair follicle morphogenesis (Paus and Cotsarelis 1999; Botchkarev and Paus 2003). Remarkably, several signaling pathways that drive and follow mesenchymal–epithelial interactions during hair follicle morphogenesis are reused during the hair growth cycle (cf. Chuong 1998). While hair growth cycle dynamics vary somewhat between different mammalian species (see Section 1.3.3), between different body sites, and even between different hair follicle subtypes in the same body site, there exists an intrinsic rhythmic activity that can be modulated not only by systemic factors but more importantly also by the hair follicle's own locally directed activities.

1.2.1.2 Catagen

Catagen has been described by Ralf Paus as a "partial organ suicide" whereby the growing terminal hair follicle is reduced by more than two-thirds of its growing tissue mass (Figs 1.2.1 and 1.2.2). This massive cell death is both controlled and rapid (about 2–3 weeks on human scalp and 3–5 days in mice) and involves substantial remodeling not only of the hair follicle itself (Lindner et al. 1997), but also the surrounding dermis.