

# Towards Defining the Pathogenesis of the *Hairless* Phenotype

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**Mutation of the *hairless* (*hr*) gene in mice causes severe abnormalities during the first hair follicle regression (catagen), resulting in complete baldness. Here, we further characterize how hairlessness develops in HRS/J *hairless* mouse skin (*hr*) by histology, histochemistry, immunohistology, and *in situ* hybridization. We show that, in *hr* skin, only two defined epithelial cell populations in the distal outer root sheath (ORS) retain their integrity, whereas the rest of the ORS disintegrates. The surviving distal ORS forms the characteristic utriculi, whereas the remnants of the bulge get isolated from other epithelial compartments, but retain the capacity to proliferate and to produce either columnar epithelial outgrowths or selected dermal cysts. Normal dermal papilla structures get lost during the development of hairlessness. Based on the patterns of keratin 17 mRNA and neural cell**

**adhesion molecule antigen expression, and on the distribution of alkaline phosphatase activity, we propose that dermal cysts in *hr* skin arise from (i) the central ORS, (ii) bulge-derived cells, or (iii) the disintegrating proximal ORS under the influence of dermal papilla remnants. The *hr* mutation seems to disrupt the integrity of key functional tissue units in the hair follicle, possibly due to a dysregulation of normal, catagen-associated apoptosis and/or an impairment of cell adhesion, whereas the distal follicle epithelium (including its stem cell region) seems to be largely protected from this. Thus, *hairless* mice offer a unique model for dissecting the as yet obscure functional properties of the *hr* gene product in maintaining follicle integrity during normal catagen. **Key words:** *apoptosis/hair follicle/keratin 17/NCAM/stem cells/trichoepithelioma.* *J Invest Dermatol* 110:902–907, 1998**

Mice with the *hairless* mutation are born with seemingly normal hair follicles (HF); however, in HRS/J mice, an inbred mouse strain carrying this mutation, complete and persistent hair loss begins to develop around 2 wk after birth, presumably after entry into the first catagen stage (Montagna *et al*, 1952), i.e., the time point when HF initiate their life-long cycles of regression (catagen), resting (telogen), and growth (anagen) (Paus, 1996; Stenn *et al*, 1996). For as yet entirely unknown reasons, the previously normal-appearing HF of these mice suddenly disintegrate, and leave behind two characteristic epithelial structures, comedo-like malformations that open onto the epidermis (so-called utriculi), and dermal cysts (DC) (Montagna *et al*, 1952; Mann, 1971). This temporal pattern of hair shedding and follicle disruption in *hr/hr* mutants suggests that the *hr* gene is involved in the controls that initiate HF cycling, i.e., in the regulation of the first anagen-catagen HF transformation.

The *hairless* phenotype in HRS/J mice is associated with an autosomal recessive allelic mutation in a gene designated *hairless* (*h*) that maps to mouse chromosome 14 (Stoye *et al*, 1988). The corresponding gene product has not yet been identified; however, based on the presence of a potential DNA-binding zinc-finger domain in the *hr* gene with

high similarity to the zinc finger regions of known transcription factors, it has been proposed that the *hr* protein also belongs to a family of transcription factors (Cachon-Gonzalez *et al*, 1994). By *in situ* hybridization, expression of the *hr* gene was localized to normal murine epidermis and HF, suggesting that the primary molecular defect in *hairless* mice is intrinsic to the skin epithelium (Cachon-Gonzalez *et al*, 1994). Furthermore, because the phenotype of the *hr* mutation in mice shows striking similarities to the rare human skin disease *papular atrichia* (Sundberg *et al*, 1989), a functional *hr* gene may also be important to normal human skin physiology.

Though the molecular basis of the *hr* mutation has become better defined, the immediate sequence of events leading to the shedding of hairs is ill-understood. Despite Montagna's pioneering work (Montagna *et al*, 1952), even the origin and pathomorphology of different epithelial structures in *hr* mouse skin remain uncertain, and a biologically convincing hypothetical scenario that explains the development of the *hr* phenotype has yet to be proposed.

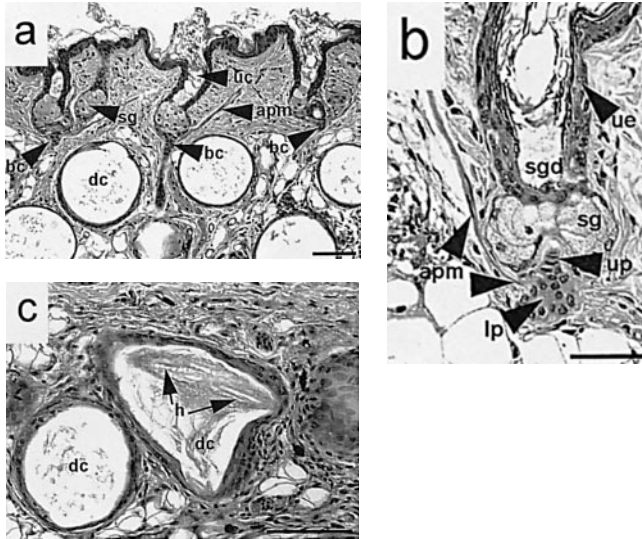
As an important step towards obtaining further insight into the molecular and cellular consequences of the *hr* gene mutation, we have re-examined the basic histopathology, and have assessed the alkaline phosphatase (AP) activity, the patterns of Ki-67 and neural cell adhesion molecule (NCAM) antigen expression, and the gene expression of murine keratin 17 (MK17) in the skin of adult *hairless* HRS/J *hr/hr* mice.

Keratin 17 was chosen for these studies because we had recently found this keratin to be a useful marker of specific functional keratinocyte units in the normal murine HF (Panteleyev *et al*, 1997a). Alkaline phosphatase activity was employed as a useful marker for dermal papilla (DP) fibroblasts (Handjiski *et al*, 1994). NCAM expression was studied for the same reason, because NCAM is expressed in normal HF in the DP, and in the perifollicular connective tissue sheath (Chuong *et al*, 1991; Kaplan and Holbrook, 1994; Combates *et al*, 1997; Müller-Röver

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Abbreviations: AP, alkaline phosphatase; DC, dermal cyst; DP, dermal papilla; HF, hair follicle; *hr*, hairless; IR, immunoreactivity; MK17, murine keratin 17; NCAM, neural cell adhesion molecule; PBDC, putative bulge-derived cells; SG, sebaceous gland.



**Figure 1. Characteristic phenomenology of *hairless* mouse skin (14–18 wk old HRS/J mice) (hematoxylin and eosin).** (a) The SG (sg) are connected with the skin surface via the utricular cavity (uc). The utricular epithelium is hyperkeratinized, whereas DC (dc) display a low level of keratinization, as evident from the lack of cornified material in the DC cavities, compared with the utriculi; apm, arrector pili muscle; bc, PBDC. (b) Close-up of the utriculi-SG-unit in *hr* skin. PBDC clusters at the proximal end of this unit are in intimate association with the arrector pili muscle (apm). They display substantial cellular diversity: the upper portion (up) is constituted of flattened cells with condensed nuclei, which are positioned in one vertical line, forming a cell column. Often, but not always, direct contact between the uppermost cells of these columns and the utricular epithelium (ue) can be appreciated (see Fig 2a). The lower portion (lp) of PBDC clusters consists of relatively undifferentiated cells with clear, swollen cytoplasm, large spherical nuclei, and round contour. (c) A hair-like structure (h) in the deep-located DC (dc). Scale bars, 100  $\mu$ m.

and Paus, 1998). Ki-67 (nuclear matrix-associated proliferation-related antigen) immunohistochemistry (Gerdes *et al*, 1997) was used to estimate constitutive proliferative activity of keratinocytes in different HF compartments of *hr* skin.

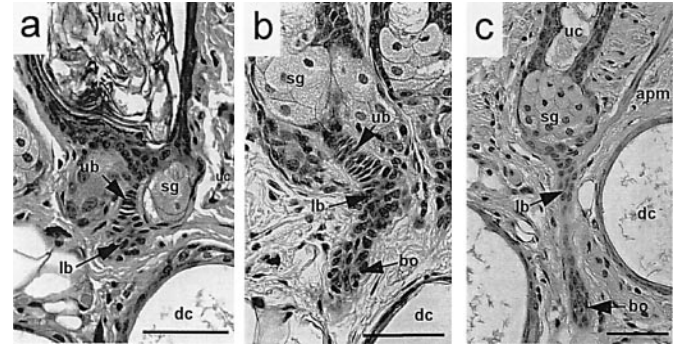
This study, which was designed as the first basic step in a more comprehensive, ongoing analysis of *hr* biology and pathology, casts new light on the morphologic consequences of the *hr* mutation, and suggests how the *hairless* phenotype may develop in *hr/hr* mice.

#### MATERIALS AND METHODS

**Animals** Female HRS/J *hairless* (*hr/hr*) mice, 14–18 wk old, were purchased from Jackson Laboratories (Bar Harbor, ME) and were housed in community cages in the Institute of Toxicology (Free University, Berlin) under standard conditions (12 h light periods, water and mouse chow *ad libitum*). Only a limited number of untreated, adult HRS/J *hr/hr* mice were available for analysis from the controls for a study addressing the effects of dioxin on *hr* mouse skin (Panteleyev *et al*, 1997b).

**Skin sections, histochemistry** The back skin of eight macroscopically *hairless hr/hr* (–/–) mice was harvested for histology as described elsewhere (Paus *et al*, 1994). Skin samples were fixed in 4% paraformaldehyde and embedded in paraffin (JUNG-Histowax, Reichert-Jung, Heidelberg, Germany) according to standard procedures. Sections of 5  $\mu$ m were mounted on silane-coated glass slides (six slides with four sections each per mouse) and were prepared for *in situ* hybridization or were stained with hematoxylin and eosin for routine histology.

**Immunohistochemistry** Additional skin samples were frozen in liquid nitrogen immediately after harvesting and were embedded in Tissue-Tek (MILES, Elkhart, IN) medium for storage at  $-70^{\circ}\text{C}$  as described (Paus *et al*, 1994). Air-dried 6  $\mu$ m cryostat sections were collected on silane-coated slides, fixed in cold acetone ( $-20^{\circ}\text{C}$ ) for 10 min, and then used for histochemical detection of alkaline phosphatase activity (Handjiski *et al*, 1994) or for immunohistochemistry. For NCAM immunohistochemistry, sections were incubated overnight (room temperature) with primary rat anti-mouse NCAM monoclonal antibodies (Boehringer, Mannheim, Germany) at a dilution of 1:100, and then treated



**Figure 2. Putative bulge cells-associated epithelial strands (bo) in the skin of *hr/hr* mice (hematoxylin and eosin).** (a) Normal structure of PBDC clusters without signs of associated epithelial strands. Note the direct connection of the utricular epithelium with the upper portion of the PBDC. (b, c) Strands of epithelial cells associated with the lower portion of PBDC clusters. See also Fig 4(e, f). ub, Upper portion of the PBDC; lb, lower portion of the PBDC; uc, utricular cavity; apm, arrector pili muscle; sg, SG; dc, DC. Scale bars, 50  $\mu$ m.

with biotin-SP-conjugated goat anti-rat IgG (Jackson Immunoresearch, West Grove, PA) at a dilution of 1:200. This was followed by incubation with avidin-biotin complex labeled by AP (Vector Laboratories, Burlingame, CA) as described (Paus *et al*, 1994). Ki-67 immunoreactivity (IR) was studied in paraffin sections (5  $\mu$ m) using rabbit anti-serum (Dianova, Hamburg, Germany) at a dilution of 1:100 (Gerdes *et al*, 1997), followed by an incubation with biotin-conjugated F(ab)2 fragment of a goat-anti-rabbit IgG (Jackson Immunoresearch; 1:200). Skin sections were then incubated with ABC complex (Vecta-Stain-Kit, Vector Laboratories).

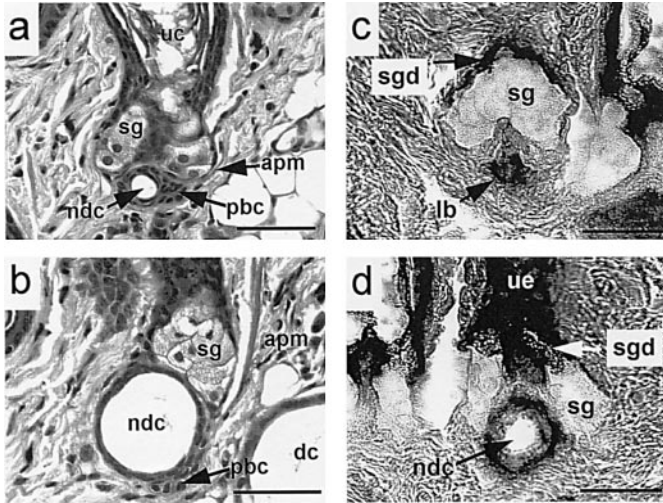
**In situ hybridization** *In situ* hybridization for MK17 expression was performed as described (Panteleyev *et al*, 1997a, b). For control purposes, the MK17 expression seen in HF of *hr* mice was compared with the MK17 expression of C57BL/6 mice with normal hair growth patterns (Panteleyev *et al*, 1997a).

#### RESULTS

**Histologic evaluation** Macroscopically, *hairless* mouse skin at the age of 3–5 mo is characterized by complete hairlessness. Microscopically, hyperkeratinized utriculi (i.e., epidermis-associated open comedones) that are connected by a short duct to the sebaceous gland (SG) are visible in these mice, together with one or two rows of fully developed DC in the reticular dermis (Montagna *et al*, 1952; Mann, 1971) (Fig 1A). Short of being able to sequentially analyze different age groups of *hr* mice, we wished to distill as many indications to the pathogenesis of the *hr* phenotype as possible from an analysis of *hr* skin during this window of postnatal HRS/J *hr/hr* life.

On closer analysis, compact clusters of epithelial cells were noted to be localized just beneath the SG (Fig 1B). In these cell clusters, substantial cellular diversity was evident. Their upper portion was surrounded by SG lobules, and consisted of cells with condensed cytoplasm and flattened nuclei, which were positioned in one vertical column, one cell just above the other (Figs 1B, 2A). The uppermost cells of these epithelial columns showed direct connections with the epithelium of the utriculi and with the SG duct, but had no other contacts with any epithelial structure (Fig 2A). The lower portion of these cell clusters was in direct contact with the insertion of the arrector pili muscle and was composed of cells with clear, swollen cytoplasm, large spherical nuclei, and round contour (Fig 1B). This proximal group of epithelial cells sometimes was connected to long downward-oriented strands of epithelial cells (Fig 2B, C). In other cases, these strands were replaced by typical DC in different developmental stages (Fig 3A, B), which usually expressed MK17 mRNA (see below; Fig 3C, D).

DC in *hr* skin were well rounded and exhibited a pattern of slow, centripetal differentiation within a thin layer of cyst epithelium (Figs 1A, 2A, C), whereas a minority of more proximally located cysts was more unevenly shaped, displayed a thicker cyst epithelium, and contained hair-like filamentous structures (Fig 1C).



**Figure 3.** Formation of a new DC in close association with PBDC (*a* and *b*, hematoxylin and eosin; *c* and *d*, MK17 mRNA *in situ* hybridization with a digoxigenin-labeled riboprobe). (*a*) Initial stage of the process (note the circular arrangement of the cells and the formation of a central cavity). (*b*) Subsequent formation of new DC (ndc) that is still connected to PBDC (pbc). (*c*) MK17 mRNA expression in the lower portion of the PBDC. (*d*) MK17 mRNA expression in the cyst, derived from the cells outlined in (*c*). Note the high expression level of MK17 mRNA in the lower portion of the utricular epithelium (ue). sg, Sebaceous gland duct; lb, lower bulge-derived cells; ndc, new DC; apm, arrector pili muscle; uc, utricular cavity; dc, DC; sg, SG. Scale bars, 50  $\mu$ m.

**Alkaline phosphatase activity** In addition to blood vessels and arrector pili muscles, which are positive for AP activity in normal murine skin (Handjiski *et al*, 1994), positive AP staining in *hr* skin was detected only in the epithelium of selected DC (Fig 4A). Neither regular AP<sup>+</sup> DP structures, nor AP<sup>+</sup> DP remnants were detectable. Instead, several AP-positive DC were seen. These were only located deep in the reticular dermis, close to the subcutis (Fig 4A, scm). Interestingly, only AP-positive DC contained hair shaft-like structures (Fig 4A, h).

**NCAM expression** Unlike normal mouse skin, which is characterized by strong NCAM-IR in the DP and myelinated skin nerves (Chuong *et al*, 1991; Kaplan and Holbrook, 1994; Botchkarev *et al*, 1997; Müller-Röver and Paus, 1998), NCAM-IR in the skin of *hr/hr* mice, with its absence of normal DP structures, was restricted to a thin layer of connective tissue directly adjacent to the epithelium of selected DC located in the mid-dermis (Fig 4B). Very intense NCAM-IR was also seen in the stroma of deep DC that contained hair shaft-like structures (Fig 4C) and were AP<sup>+</sup> as well (Fig 4A). In addition, the epithelial cell clusters located beneath the SG perform NCAM-IR in their upper portion and were surrounded by strongly NCAM<sup>+</sup> nerve bundles (Fig 4D). The epithelium of utriculi and their adjacent mesenchymal cells, as well as interfollicular dermal fibroblasts or DC located in the upper dermis, displayed no NCAM-IR.

**Ki-67 expression** In *hr* (–/–) skin, prominent Ki-67 IR was found in most basal layer keratinocytes of the utricular epithelium (Fig 4E) and in the epithelium of most DC (not shown). Contrary to the basal layer of interfollicular epidermis of *hr* and of normal mouse skin (Gerdes *et al*, 1997), where only some keratinocytes are Ki-67<sup>+</sup>, nearly all basal keratinocytes in the middle and proximal portion of the utricular epithelium were strongly Ki-67<sup>+</sup> (Fig 4E). This was the case in most follicular remnants, though not in all (Fig 4G). Ki-67 IR was also detectable in 10–15% of downward-oriented epithelial strands that were occasionally seen beneath the SG (Fig 4E, F), attesting to ongoing cell division in this cell population. In addition, the new DC that formed in the region of the arrector pili muscle attachment also exhibited Ki-67<sup>+</sup> cells (Fig 4G), thus demonstrating high proliferative activity.

**MK17 expression** IR denoting MK17 mRNA transcripts was exclusively localized to the epithelial remnants of *hr* HF. In utricule epithelium, MK17-mRNA expression was detected mainly in the inner cell layers adjacent to the utricular cavity (Fig 4I). MK17 staining was seen in the keratinized part of the utricular epithelium as well as in the nonkeratinized proximal part, which corresponds to the SG duct of normal hair follicles. Neither the SG epithelium, nor the more distally located part of the utricles, showed MK17 mRNA expression (Fig 4I). The epithelium of the DC expressed MK17 mRNA to a similar degree as the utricule epithelium (Fig 4I). The MK17 signal was evenly distributed in the epithelium of all DC. Individual sebocytes that were occasionally visible within the DC epithelium were MK17 negative (not shown).

The compact cell clusters localized proximal to the SG and in association with arrector pili muscle fibers [i.e., putative bulge-derived cells (PBDC)], were normally negative or, at the most, weakly positive for MK17 transcripts; however, whenever these cell clusters were associated with new growing DC (Fig 3A, B), their proximal portion exhibited high levels of MK17 mRNA-IR (Fig 3C, D).

Hybridization of representative skin sections with MK17 sense-probes as negative controls showed no specific IR (not shown).

## DISCUSSION

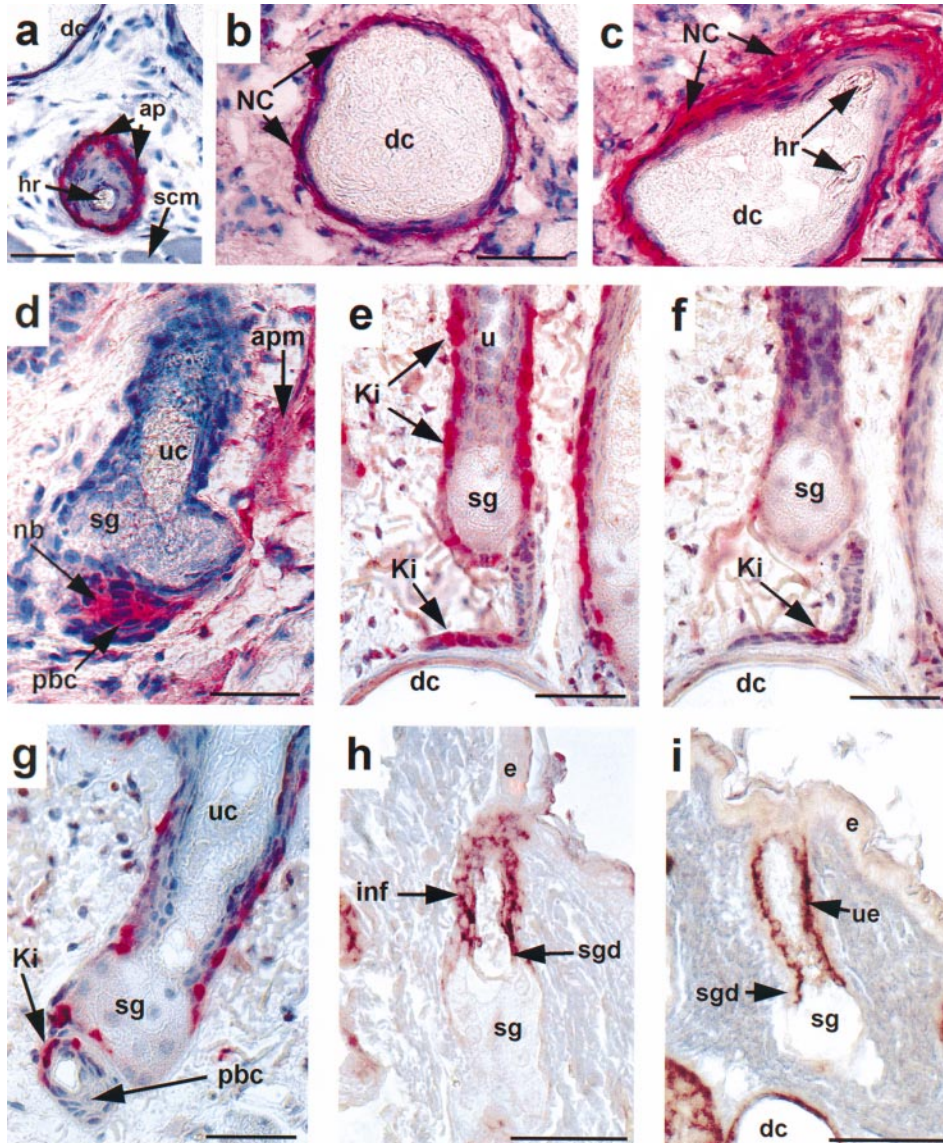
The origin of utricles and DC in *hr* mouse skin has so far been uncertain (Montagna *et al*, 1952; Sundberg, 1994). Here, we show that the MK17 expression pattern in utricles (Fig 4I) resembles the one observed in C57BL/6 mice with their normal hair coat (Fig 4H), where the infundibular outer root sheath (ORS) constantly expresses MK17 mRNA (Panteleyev *et al*, 1997a). This similarity in MK17 expression, and the location of the SG proximal to the utricule epithelium, strongly supports the concept that the utricles in *hr* skin develop from the infundibular ORS, rather than from other parts of the normal hair follicle epithelium (Fig 5).

The location of the compact clusters of relatively undifferentiated epithelial cells beneath the SG (Fig 1A), and their association with arrector pili muscle fibers (Fig 1A, B), suggest that these cells correspond to, and derive from, the bulge, which contains the key epithelial stem cells of the normal HF (Cotsarelis *et al*, 1990). Based on morphologic evidence (Figs 2A–C, 4E, F), these cells are not a part of the utricular epithelium, from which they are well distinguishable (Fig 1B), neither are they “DP-like cells” (Sundberg *et al*, 1991), because MK17 expression (Fig 3C) strongly confirms their epithelial nature. It is important to note that the PBDC in *hr* mouse skin identified here represent an isolated cell conglomerate, whereas bulge cells in normal HF are deeply embedded into a protrusion of the distal ORS (Cotsarelis *et al*, 1990). This segregation of PBDC from the specific bulge-containing portion of the distal ORS is confirmed by their close association with palisade nerve fibers (Fig 4D), which form a specific network around the isthmus region of the normal HF (Botchkarev *et al*, 1997).

It has previously been proposed that widening of the follicular canal, improper hair club formation, and/or follicular keratosis are the main reasons for hair loss in *hr* skin (David, 1934; Fraser, 1946; Mann, 1971). The homology of the utricles solely to the infundibular portion of the normal ORS that we demonstrate here (Fig 4H, I), and the isolated position of the PBDC in *hr* mouse skin (Fig 1B), suggest not only that the cycling, proximal part of the HF epithelium disintegrates as a result of the *hr* mutation, but also that the permanent, distal portion of the HF ORS (isthmus) is destroyed (Fig 5). Thereby, the hair shafts formed during the course of HF morphogenesis may loosen their mooring in the ORS and fall out, resulting in alopecia. In addition, the DP gets disconnected from the remnants of the HF epithelium (utriculi and bulge-derived cells). This loss of normal epithelial–mesenchymal interactions, in turn, likely disrupts the capacity of HF remnants to cycle.

Though this awaits confirmation, it is reasonable to speculate that this disintegration of the permanent ORS portion results from a dysregulation of the massive, yet tightly controlled keratinocyte apoptosis, which normally occurs in well-defined regions of the follicle





**Figure 4. Histochemical (a, AP activity), immunohistochemical (b-d, NCAM; e-g, Ki-67) staining, and *in situ* hybridization (h, i, MK17 riboprobe) of hairless mouse skin (14–18 wk old *hr/hr* HRS/J mice).** Scale bars: a–g, 50  $\mu$ m; h, i, 100  $\mu$ m. (a) AP<sup>+</sup> DC in the deep dermis. Note the location of these AP<sup>+</sup> (ap) cysts below the majority of AP DC and near to the subcutaneous muscle layer (scm), the panniculus carnosus. Most of these cysts contain hair fragments (hr), suggesting their origin from abnormal secondary hair folliculoids. (b) NCAM-IR in DC located in the mid-dermis. The NCAM<sup>+</sup> cells (NC) form a thin sheath around the DC (dc) epithelium, resembling the perifollicular connective tissue sheath of normal hair follicles. (c) NCAM-IR (NC) in DC (dc) containing hair remnants (hr). (d) NCAM-IR in the PBDC in *hr* mouse skin. The NCAM<sup>+</sup> longitudinal nerve bundles (nb) surround the upper portion of the PBDC. (e–f) Ki-67 expression on two serial sections of the proximal part of the utriculi-SG unit. Ki-67<sup>+</sup> cells (Ki) are visible in the utricular epithelium and in the downward-oriented epithelial strand that is associated with PBDC. (g) Ki-67 expression (Ki) in the new DC epithelium, which is formed in close association with PBDC (pbc). (h, i) MK17 mRNA expression (*in situ* hybridization with a digoxigenin-labeled riboprobe) in the hair follicle infundibulum of C57BL/6 mice with their normal hair coat and cycle (H), and in the utricular epithelium of hairless mice (i). Note the localization of MK17 transcripts to the innermost cell layers of the utricular (ue) and infundibular (inf) epithelium, and the presence of MK17 mRNA in SG ducts (sgd), but not in the SG epithelium in both normal (h) and *hr* (i) mice. e, Epidermis; dc, DC; sg, SG.

epithelium during catagen (Weedon and Strutton, 1981; Lindner *et al.*, 1997). We failed to detect an upregulation of TUNEL<sup>+</sup> cells in the HF remnants of 3–4 mo old *hr/hr* mice (Panteleyev and Paus, unpublished observation). Because HF degeneration by excessive apoptosis would be expected to occur between the fourteenth and twentieth days of age, and would long have been completed weeks thereafter, this negative result is not at all surprising. The challenge now is to subject *hr/hr* mice of this age group to TUNEL.

In addition to apoptosis dysregulation, the abnormalities in NCAM expression revealed in *hr* skin (Fig 4C) suggest that a collapse of normal HF topobiology, i.e., the shaping and maintenance of follicle morphogenesis and structure by cell adhesion molecules (Chuong *et al.*, 1991; Kaplan and Holbrook, 1994; Müller-Röver and Paus, 1998), may contribute to HF disintegration in *hr* skin. Does the *hr* protein, therefore, represent a transcription factor that normally controls apoptosis and coordinates cell adhesion molecule expression in defined populations of ORS keratinocytes? Interestingly, the remnants of both the HF infundibulum (utriculi) and the PBDC are protected from the – as yet unknown – consequences of lacking the *hr* gene product, whereas DP- and other ORS-derived cells are not. Still, ORS keratinocytes are not completely extinguished, as evidenced by the formation of various classes of DC by these cells.

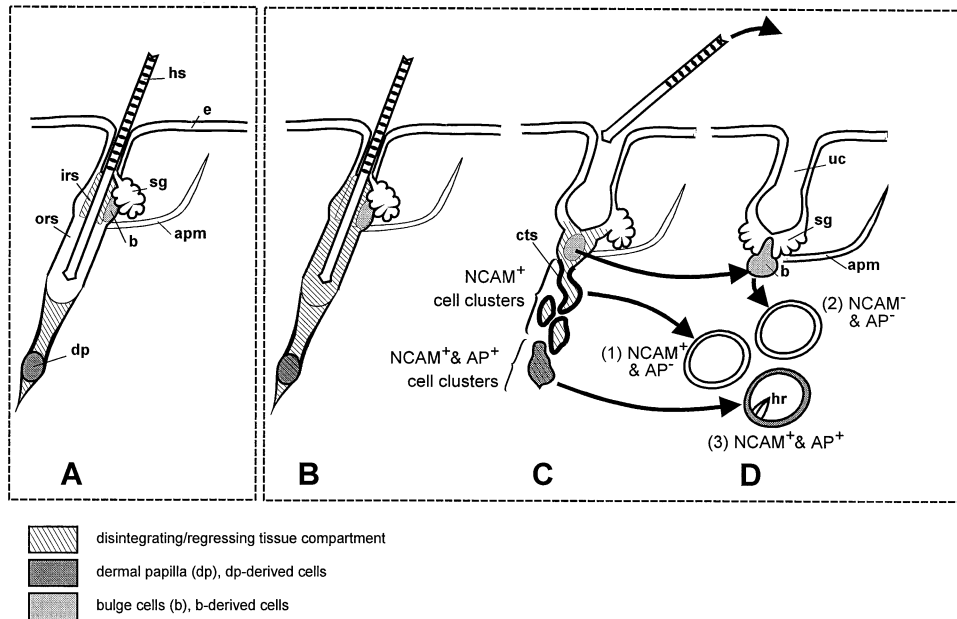
The morphologic structure, location (Fig 2), and proliferation activity (Fig 4E, F) of epithelial strands that are in intimate association with the clusters of PBDC are reminiscent of the downward growth

of epithelial cells during early anagen of the normal hair cycle (cf. Paus, 1996; Stenn *et al.*, 1996). Therefore, the downward-oriented epithelial strands in *hr* skin may represent outgrowths of the PBDC.

We show that the PBDC in *hr* skin also have the capacity to produce DC (Figs 3A–D, 4G). These cysts are quite similar to trichoepitheliomas, which are thought to be produced by an abnormal proliferation of bulge cells (Pinkus, 1951). Therefore, *hr* skin might offer a useful model for studying the mechanisms of trichoepithelioma induction.

Which signals may induce the growth of cells in the PBDC of *hr* mouse skin? In normal HF, the role of the DP in the generation of such a signal is widely accepted (Jahoda *et al.*, 1984; Cotsarelis *et al.*, 1990; Paus, 1996; Stenn *et al.*, 1996). Because AP activity and NCAM-IR are characteristic markers for normal DP fibroblasts (Chuong *et al.*, 1991; Handjiski *et al.*, 1994; Kaplan and Holbrook, 1994; Combates *et al.*, 1997), the absence of AP<sup>+</sup> and NCAM<sup>+</sup> cells in the proximity of the PBDC in *hr* skin suggests that DP-derived signals are *not* essential for inducing the outgrowth of these cells. This raises the possibility that cyclic growth activity is generated from within this particular epithelial cell population, independent of inductive signals from a specialized mesenchyme. The lack of appropriate survival signals from a specialized mesenchyme (Raff *et al.*, 1993) in *hr* skin, however, may be a limiting factor for this proposed, autoinduced bulge cell proliferation.

Although regular DP structures are absent in the skin of *hairless*



**Figure 5. Proposed scenario of hairless skin pathogenesis.** (A) Late catagen (catagen VII according to Straile *et al*, 1961) in normal hair follicle of C57BL/6 mice. Only the cycling portion (o) of the hair follicle epithelium is regressing, and the DP does not disintegrate. hs, Hair shaft; e, epidermis; sg, SG; b, bulge; irs, inner root sheath; ors, ORS; apm, arrector pili muscle; dp, DP. (B) Late catagen in *hr/hr* hair follicles. The entire ORS, except for infundibulum and bulge, disintegrates. The ORS disintegration, particularly in the isthmus region, makes the hair shaft, which has been generated during follicle morphogenesis, to loose its mooring in the follicular canal. (C) Hypothetical intermediary stage of the hair follicle disintegration as a consequence of the *hr* mutation. The ORS is degraded, whereas infundibulum, SG, and bulge cells partially retain their integrity. Clusters of disintegrated central and proximal ORS keratinocytes associate with NCAM<sup>+</sup> remnants of the former connective tissue sheath (cts). The most proximal portion of these cells (AP<sup>+</sup> cell clusters) represents remnants of the DP. (D) Structure of adult *hairless* mouse skin. Utricle (uc) originate solely from the infundibular portion of the ORS. The bulge-derived cells (b) retain their integrity, but get separated from the proximal follicle epithelium. DC are then formed from three different sources: (i) parts of the disintegrated ORS (NCAM<sup>+</sup>, AP<sup>+</sup> stroma); (ii) spontaneous growth activity of PBDC (NCAM<sup>+</sup>, AP<sup>+</sup> stroma); (iii) AP<sup>+</sup>, NCAM<sup>+</sup> epithelial remnants of the ORS, possibly in conjunction with DP remnants, which may form epithelial-mesenchymal interaction units that retain the capacity to form incomplete, hair-like structures (h). sg, SG; apm, arrector pili muscle.

mice, we here describe ectopic AP activity associated with NCAM-IR in the stroma of selected DC in the reticular dermis (Fig 4A, C). The observation that only these AP<sup>+</sup> and NCAM<sup>+</sup> DC contain hair-like structures (Fig 1C) suggests that they originate from HF remnants that retain (at least in part) the ability to produce incomplete hair shafts, perhaps due to the presence of morphogen-secreting AP<sup>+</sup> and NCAM<sup>+</sup> remnants of disintegrated DP fibroblast populations (Fig 5).

The origin of DC in the skin of *hr* mice is a subject of much debate. Formerly, it had been suggested that they originate, at least in part, from SG (Crew and Mirskaia, 1931; Montagna *et al*, 1952); however, several lines of evidence strongly suggest a nonsebaceous origin of DC in *hr* skin: DC are present even in the skin of double-homozygous *hairless* and *asebia* mice (*hr/hr*, *ab/ab*) that are characterized by a complete absence of SG (Gates *et al*, 1969). Also, the NCAM<sup>+</sup> sheath of DC in *hr* skin (Fig 4B) resembles the NCAM expression of the perifollicular connective tissue sheath of normal HF (Chuong *et al*, 1991), supporting that DC derive from the ORS. Finally, MK17 mRNA expression was detected in the epithelium of all DC (Fig 4I), but never in SG, neither in *hairless* skin (Fig 4I) nor in normal C57BL/6 mice (Fig 4H) (Panteleyev *et al*, 1997a), nor in humans.<sup>1</sup>

Because murine HF with normal growth and cycling express MK17 in the innermost cell layer of the proximal ORS (Panteleyev *et al*, 1997a) that is normally surrounded by a NCAM<sup>+</sup> connective tissue sheath (Chuong *et al*, 1991), the MK17<sup>+</sup> DC epithelium of *hr* mice surrounded by thin NCAM<sup>+</sup> connective tissue sheath most likely arises from remnants of the disintegrating, proximal ORS (cf. Bernerd *et al*, 1996). This is further supported by the finding that clusters of proximal ORS keratinocytes from human anagen HF cultured under three-dimensional conditions can form spheroid structures with centripetal differentiation (Limat *et al*, 1994), not unlike the DC of *hr* mice.

Taken together, this suggests three possible pathways of DC morphogenesis in the skin of *hr* mice (Fig 5): (i) from the disintegrating ORS, resulting in cysts positioned in the central dermis that display an NCAM<sup>+</sup> connective tissue sheath; (ii) from PBDC, resulting in AP<sup>+</sup>, NCAM<sup>+</sup> cysts positioned in the upper dermis; (iii) from as yet ill-characterized epithelial structures that are located deep in the dermis (perhaps in conjunction with surviving AP<sup>+</sup> and NCAM<sup>+</sup> DP fibroblasts), and that retain the capacity to form incomplete hair-like structures. These different pathways of DC morphogenesis would help to explain the long-appreciated pluripotency of the DC epithelium in *hr* mice, which is capable of lipogenesis, squamous differentiation (Montagna *et al*, 1952; Sundberg, 1994), and the formation of hair-like structures, corresponding to the distinct pathways of epithelial differentiation seen in normal HF (Pinkus *et al*, 1981).

Thus we propose that one of the main cellular mechanisms of hair loss in *hairless* mice, and the main reason for the subsequent failure of hair follicle cycling, is the disintegration of most of the upper ORS due to loss of the *hr* gene product (Fig 5), a putative transcription factor (Cachon-Gonzalez *et al*, 1994). This may cause profound alterations in the transcription of genes involved in the control of ORS keratinocyte apoptosis and of follicle topobiology (cf. Lindner *et al*, 1997; Müller-Röver and Paus, 1998).

The improved understanding of the histogenesis of the *hairless* phenotype that this study provides, invites one to exploit the *hr* mutation as a unique model for dissecting the interactions of bulge and DP as well as the transcriptional control of HF regression under physiologic and pathologic conditions. Given that premature or retarded HF regression is pivotal to the development of most common hair growth disorders seen in man (Paus, 1996), analysis of the molecular and cellular pathology of the *hr* mutation may provide critical pointers to the elusive key genes of catagen control (cf. Paus, 1996; Stenn *et al*, 1996), and to more efficient means for manipulating their expression during the treatment of human hair growth disorders.

<sup>1</sup> Holland DB, Roberts SG, Cunliffe WJ: Localization of keratin 17 mRNA in acne. *J Invest Dermatol* 108:384, 1997 (abstr.)

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