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The disease occurs most commonly in female mice 6 months of age and older and in male mice that are 12 months of age and more [1]. We established a colony, starting with affected breeders, where the incidence is now 6.3% in male and 14.4% in female mice, by 18 months of age. Crosses were set up to identify genes involved in the disease that seems to be controlled by at least one dominant or semi-dominant gene with reduced penetrance.

Affected C3H/HeJ mice can be first recognized by a diffuse ventral alopecia that starts initially on the medial aspects of the proximal portion of the rear legs [1]. Alopecia of the dorsal truncal skin develops as circular foci that can be solitary or multifocal. These foci wax and wane. Approximately 17% progress to generalized alopecia [1]. Therefore, mice can present a variety of lesions ranging from focal alopecia, large areas of alopecia that appear to involve hair cycle waves, a generalized thinning of the hair coat, to diffuse alopecia [2].

Microscopically, all hair follicle types [2] are affected. Only follicles in anagen are found with a severe mixed inflammatory cell (predominantly lymphocytic) infiltrate in and around the follicles. The isthmus and to a lesser degree the bulb regions are primarily involved. Intrafollicular migration of lymphocytes is associated with disruption of the follicular sheaths, focal necrosis with separation at the junction of the dermal papilla and matrix cells, and structural changes in the hair shaft resulting in focal destruction with loss of pigment [1,2]. Scanning electron microscopic studies of hairs have revealed focal degenerative changes including longitudinal fissures, loss of cuticle, flattening, and breakage with splintering of the hair shafts. The alopecia is due to breaking off of hair shafts as they emerge from the skin.

The lymphocytic infiltrate in and around hair follicles consists predominantly of $CD8^+$ lymphocytes and smaller numbers of $CD4^+$ cells. These can be eliminated by intralesional steroid injections resulting in regrowth of the hair [1].

C3H/HeJ mice are readily available from production colonies at The Jackson Laboratory. However, these mice rarely have alopecia areata at the age of shipment. Mice have to be aged and followed to determine which will develop the disease.

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Fibroblast Growth Factor and the Hair Cycle of the Hairless Mouse

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Hair follicles arise in developing skin from complex cellular interactions involving molecules such as fibroblast growth factor (FGF). Immunolocalization of acidic and basic FGF (FGF-1 and FGF-2), particularly around the basement membrane zone (BMZ) during follicle development, demonstrates that these growth factors may have related functions in local tissue remodeling during follicle morphogenesis, and later may serve to regulate cellular mitotic activity and differentiation in the follicle [1].

In developing skin, FGF-2 high-affinity receptors are found on cells of the dermal papilla as well as on the basal keratinocytes of the epidermis and on follicle bulb cells [2]. Heparan sulfate proteoglycan molecules, which are components of basement membranes and extracellular matrix, appear to be low-affinity receptors for FGF-2; initial binding of FGF-2 to heparan sulfate proteoglycan molecules is apparently essential for presentation of the growth factor to its high-affinity receptors. FGF-2, when injected into newborn mouse skin, affects both the initiation and development of hair follicles, and subsequently delays the hair cycle [3]. The mechanism of action of FGF-2 in these processes is unknown, and little is known about FGF-2 in relation to the hair cycle. Thus, this study was undertaken to examine the role of FGF-2 in the hair cycle by examining FGF-2 expression in the skin and hair follicles of the hairless (*hr*) mutant mouse, particularly during the transition stages when substantial morphologic changes are occurring.

The hairless mouse initially develops a full hair coat but, between the ages of 10–14 d, begins to lose hair on the face and legs. This continues until the whole head is bare. Shedding proceeds in a cephalo-caudal direction until the animal is completely naked by about 21 d of age [4]. There is little subsequent regeneration of the hair. The hair loss and lack of regrowth appears to be related to several events taking place during the catagen phase of the hair cycle. Incomplete formation of the club-hair at catagen and excessive widening of the pilary canal result in premature shedding of the

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hair, whereas subsequent generations of hair have difficulty forming because the follicles are distorted and the distal end can become separated from the rest of the follicle [5].

Skin samples were obtained from the dorsal region of hairless mutant mice and unaffected littermates; the samples were fixed and paraffin embedded. Tissue sections (5 μm) were deparaffinized, then probed with a polyclonal antibody against FGF-2 (a kind gift of Dr. M. Reidy, University of Washington, Seattle, WA). A biotinylated goat anti-rabbit IgG antibody (Cappel) was applied, then the sections incubated with a streptavidin-peroxidase complex (Zymed Laboratories, Inc.). The sections were developed in 3,3′-diaminobenzidine before counterstaining with hematoxylin and mounting.

Until 10–14 d of age, mutant animals cannot be distinguished from their normal littermates. In newborn skin, FGF-2 expression was found in the basement membrane at the dermal-epidermal junction and in basement membrane surrounding the dermal papillae and the follicles themselves; staining was also found in the dermis just beneath the dermal-epidermal junction. Control sections showed no immunoreactivity. At 17 d of age, the hair follicles of mutant and normal mice were in catagen, mutant follicles becoming very distorted. FGF-2 immunoreactivity remained associated with follicle basement membrane but was absent from the basement membrane surrounding the dermal papillae, which were no longer incorporated at the bases of the follicles. The expression pattern of FGF-2 did not change during the telogen phase of the hair cycle in mutant or normal mice (20 d of age). At this stage, the hair follicles of the mutant mice were very distorted and many dermal papillae were observed to be stranded in the dermis. By 28 d of age, the follicles of the hairless mice remained in telogen whereas those of their normal littermates had entered anagen of the next cycle. FGF-2 expression did not change in the skin of mutant mice but FGF-2 immunoreactivity was again observed in dermal papillae basement membrane of normal mouse skin. No distinct differences were noted between FGF-2 expression in hair follicles at different stages of the hair cycle of the hairless mutant mice and their normal littermates.

Although FGF-2 is known to be a potent mitogen, its localization to the basement membrane of skin and hair follicles remains unclear, particularly because uncontrolled growth is not observed physiologically in the adult. We therefore hypothesize that FGF-2 is sequestered and held in a latent form by specific heparan sulfate proteoglycan molecules in basement membrane of the skin and follicles. It is then released in a controlled fashion to receptive epidermal cells to initiate proliferation and the invasive processes that occur during skin development and during the hair cycle. This hypothesis could explain why differences in FGF-2 expression were not observed between hairless mutants and their normal littermates, because the technique used in this study is not able to distinguish latent basement membrane–bound FGF-2 from released, active FGF-2. Further study of the interactions between FGF-2 and heparan sulfate proteoglycan molecules in the skin is clearly required before the specific role of FGF-2 can be elucidated.

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Loss of Vascular Endothelial Growth Factor in Human Alopecia Hair Follicles

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Vascular endothelial growth factor (VEGF) is the normal product of a single gene that appears to be activated principally by stimuli that are mitogenic for many cell types. VEGF exists in one of four different isoforms as a homodimer of disulfide-linked polypeptide chains, each of which may be 121, 165, 189, or 206 amino acids long. The various polypeptide lengths result from alternative splicing during transcription of the VEGF gene which contains eight exons; the 165 amino-acid isoform is most commonly observed. The $VEGF_{121}$ and $VEGF_{165}$ isoforms are secreted in soluble forms from producing cells; the $VEGF_{189}$ and $VEGF_{206}$ forms are less soluble because they contain increasing proportions of the hydrophobic carboxy-terminus of the molecule and after secretion tend to associate with lipid membrane components.

VEGF is an endothelial cell (EC) specific molecule binding to either *c-flt* or KDR receptor tyrosine kinases expressed exclu-

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Abbreviations: rtk, receptor tyrosine kinase; VEGF, vascular endothelial growth factor.

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