

sequences with oligonucleotide hybridization or PCR sequencing¹⁶.

Low accuracy sequence can be a step towards acquiring highly accurate data, by its use for probe generation, primer-directed strategies, or PCR sequencing. Low accuracy (1% error rate) cDNA sequence databases could also play a useful role in the identification of coding regions by homology search. Extending the length of the sequence and improving its accuracy will be necessary if such cDNA sequence databases are to be used as the basis for the analysis of conceptually translated protein sequence. The usefulness of sequence databases for purposes such as this will be determined by the quality of the data they contain.

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The first hair follicles are formed from the ectoderm, an epithelial layer that will give rise to the epidermis, and the underlying mesoderm, a mesenchymal layer that will form the dermis. Figure 1 indicates the main stages of follicle development as seen on the back of a mouse, but is representative of hair follicles in most mammals. The numbers assigned to stages will be used in this review. Melanoblasts, of neural crest origin, are usually present among the epithelial cells at the beginning of this period, and will differentiate into melanocytes in the base of the follicle and transfer pigment to the hair. Since they have relatively little influence on follicle development, they are not included in this review.

In most mammals the follicles that produce the pelage hairs – which form the coat of fur, hair or wool – begin to form in the skin during prenatal life at one location, such as the crown of the head, and extend in a wave-like manner over the body surface. In animals with a dense coat this first wave may be followed by further waves of smaller follicles producing smaller hairs. These follicles are arranged in regular patterns, usually in groups with one large primary follicle flanked by two slightly smaller ones, and a group of secondary follicles associated with each trio^{1,2}. Whisker (vibrissa) follicles develop earlier, in restricted locations, and have their own regular pattern, for example, in rows on the upper lip.

Since both pelage hair and vibrissa follicles can form *de novo* from organ cultured fragments of embryonic skin, and hairs are produced from them³, this process does not require ongoing neural or humoral signals. The early pattern of the hair follicles formed *in vitro* corresponds with the pattern observed *in vivo*.

The secret life of the hair follicle

MARGARET H. HARDY

The mammalian hair follicle is a treasure waiting to be discovered by more molecular geneticists. How can a tiny cluster of apparently uniform epithelial cells, adjacent to a tiny cluster of uniform mesenchymal cells, give rise to five or six concentric cylinders, each of which is composed of cells of a distinctive type that synthesize their own distinctive set of proteins? There is now evidence that several growth factors, cell adhesion molecules and other molecules play important roles in the regulation of this minute organ.

Similar development of feathers and/or scales can occur in cultures or grafts of embryonic skin of birds or lizards⁴. Developmental biologists have taken advantage of these features to analyse the roles of epithelium and mesenchyme in the formation of skin appendages⁵. Experiments in which the epidermis and dermis were separated in skin taken from different body regions of embryonic mice, chicks and lizards, and recombined in various ways as explants or chick chorioallantoic membrane grafts, revealed a great deal about the content of the messages that pass from one tissue to the other. However, the physical or chemical signals that convey the messages were unknown.

Figure 2 summarizes the messages that have been discovered for hair follicle differentiation in mammals. The dermal mesenchyme, separated from the back of

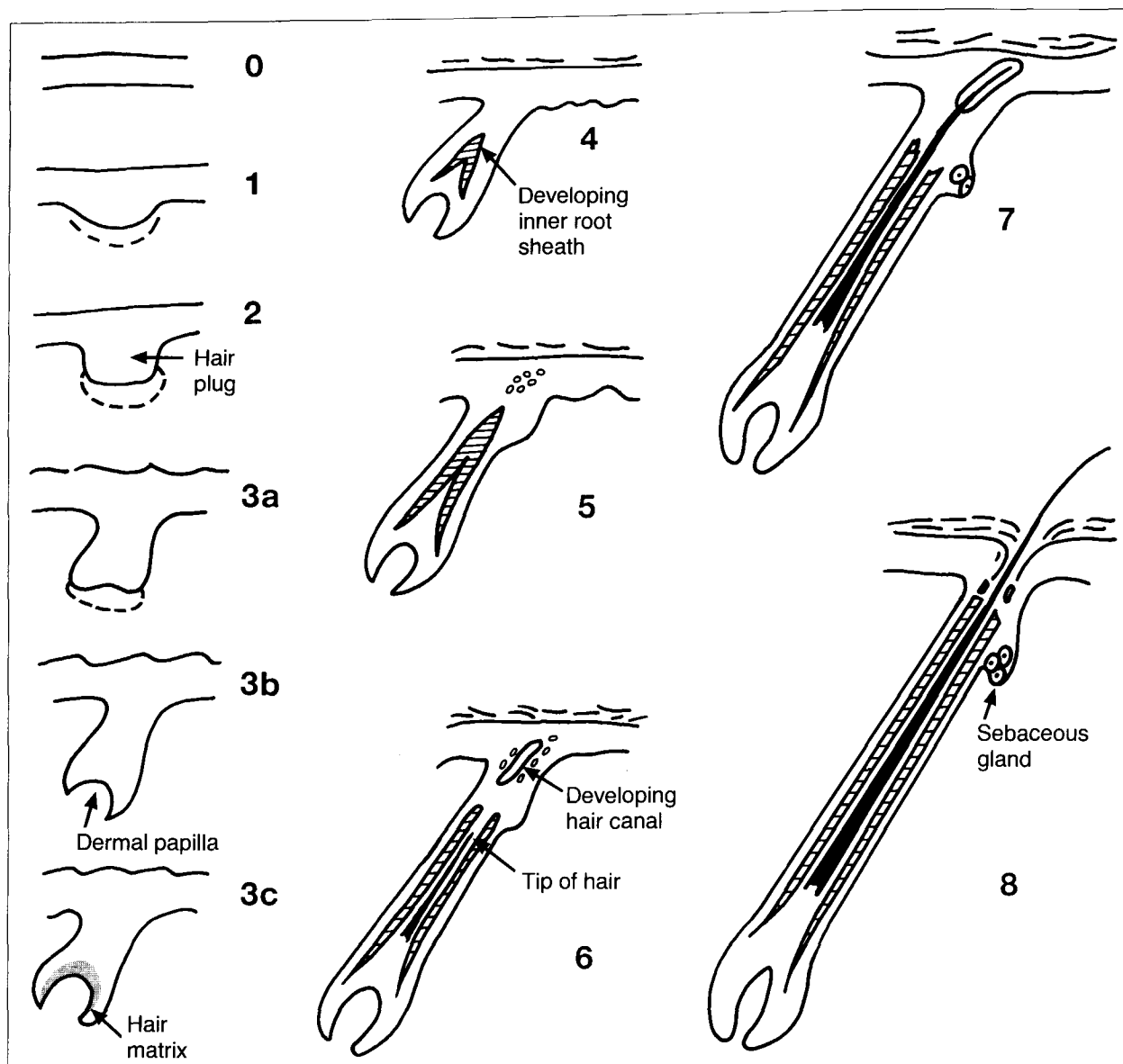


FIG 1

Stages of hair follicle development in the mouse. Solid lines outline the populations of epidermal cells, and the epithelial cells of the follicle that grow down from the epidermis. Dashed lines enclose the mesenchymal cell aggregation that becomes the dermal papilla in stages 3b, c, etc. A canal for the exit of the growing hair is outlined where it is forming in stages 6 and 7. A sebaceous gland containing rounded cells is shown growing from one side of the follicle at stages 7 and 8. The filled central rod indicates fully keratinized hair; the hatched area indicates hardened inner root sheath.

a mouse that would normally grow hairs, can form cell aggregations and then initiate thickenings (placodes), then downgrowths (plugs; Fig. 1, stage 2), from an overlying mouse epidermis, even if that epidermis is from a hair-free region such as the footpad. This is a response to the first dermal message. Mouse dermis can also initiate feather buds from chick foot epidermis, or scale placodes from lizard epidermis. So the message is 'make appendages here', and the epidermis responds by beginning to form a structure that is appropriate to its class of origin.

Less is known about the epidermal message, which is believed to pass from the mouse epithelial cells forming the follicle bud to the adjacent cluster of mouse dermal mesenchymal cells that become surrounded by the expanding base of the hair follicle

plug to form the dermal papilla (Fig. 1, stage 3b). Tissue recombination experiments have shown that only mouse dermal cells will respond to this message from a mouse hair follicle plug⁵. Thus the message might be 'make a dermal papilla', and might depend on selective cell adhesion.

The second dermal message is then transmitted from the dermal papilla to the adjacent epithelial cells of the hair plug which are then known as the 'hair matrix' (Fig. 1, stage 3c), stimulating them to divide rapidly. The hair matrix daughter cells then move up the follicle, differentiating into either hair cells (three types) or inner root sheath cells (three types), depending upon their position in relation to the longitudinal axis of the follicle (Fig. 3). Eventually a keratinized hair emerges (Fig. 1, stage 8), with a thin outer cuticle, a thick cortex and

(sometimes) a central medulla containing air spaces. In contrast, the feather buds or scale placodes that can be induced in chick or lizard epidermis by the first dermal message from mouse dermis do not grow or differentiate further⁵. Thus the second dermal message from mammalian skin is specific, 'make a hair follicle', and cannot be interpreted by epithelial cells from other classes of vertebrates⁴.

Cyclic growth of hairs, and waves of new growth

Once established, hair growth is cyclic, with every hair follicle proceeding from an active phase (anagen) through a regression and shortening phase (catagen) to a resting phase (telogen). In the greatly shortened telogen follicles of the mouse the deepest epithelial cells, constituting the 'hair germ' below the sebaceous gland opening, are undifferentiated and nondividing outer root sheath cells⁶. Below these are the dormant cells of the dermal papilla, which had moved up as the follicle regressed (Fig. 4). After telogen, the follicle is reactivated from the base of the resting structure. Some of the hair germ cells extend deeper in the form of a plug, as if responding to a new 'first dermal message' from the dermal papilla cells. As in the first cycle, the deepest hair plug cells surround the dermal papilla cells; then, as if responding to a new second dermal message, the adjacent cells, now recognized as hair matrix cells, begin to divide and give rise to the hair and inner root sheath cells, which grow into the mouth of the old follicle (Fig. 4). The old hair is eventually shed.

Thus the same sequence of messages from the dermal papilla cells appears to be involved with each hair cycle. By grafting of isolated adult rat whisker dermal papillae or cultured papilla cells, it was shown that new follicles and hairs can also be initiated from outer root sheath cells taken from the lateral wall of a whisker follicle, or even from the epidermis^{7,8}. Therefore, some other cells of ectodermal origin can become hair matrix cells after interactions with dermal papillae. A recent isotope labelling study⁹ supports the earlier interpretation from ultrastructural studies⁶ that the hair matrix cells of a new cycle in the mouse are not the former matrix cells revitalized, but derivatives of a group of late-replicating outer root sheath cells below the sebaceous glands that become part of the new hair plug⁹. It was concluded that the stem cells for the hair matrix of second and subsequent hair cycles reside in a special part of the outer root sheath, which may be represented by the 'wulst', or bulge, in the human hair follicle⁹.

In small rodents such as the mouse and rat, cycles of pelage hair growth occur several times a year¹⁰. Most wild mammals have seasonal cycles related to daylight length and endocrine activity. In wild animals, however, there are frequently differences between the cycles of the larger primary, hair-producing follicles and the smaller secondary ones, which produce a

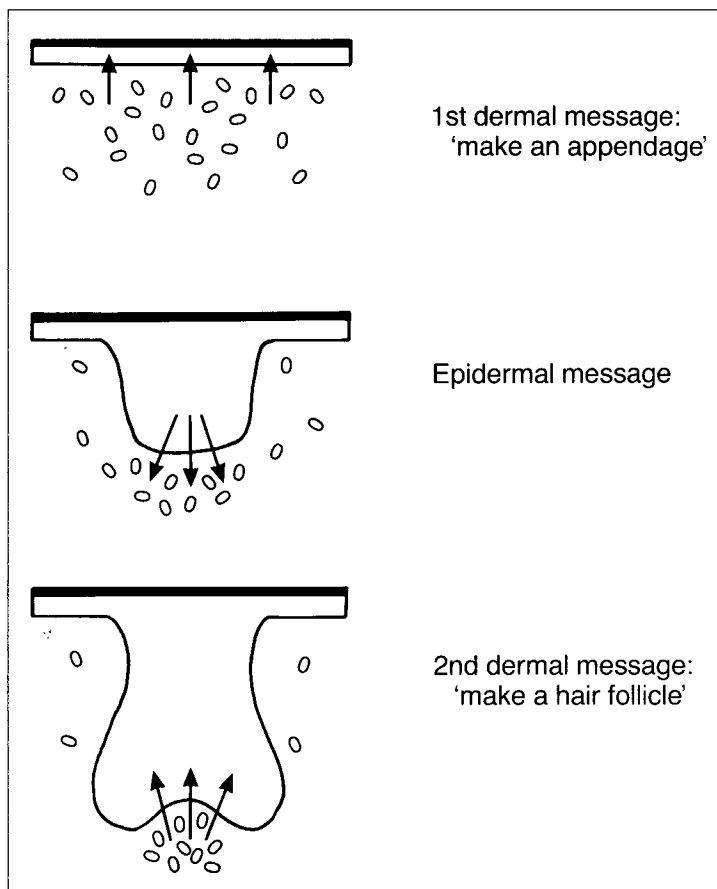


FIG 2

Epithelial-mesenchymal interactions during hair follicle development in mammals. Modified, with permission, from Ref. 36.

shorter, finer underfur. Still other species, such as guinea-pigs and humans, which may have originated in tropical regions, have a more mosaic pattern, with each follicle type within a small area following its own internal clock. Clearly, then, there must be some signals

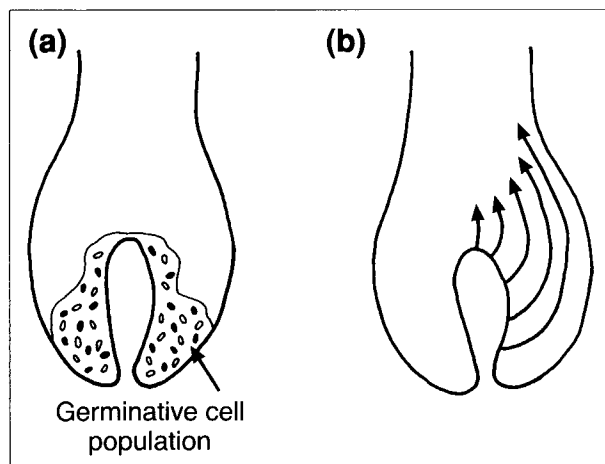


FIG 3

(a) The hair matrix region of proliferating cells is indicated in the hair bulb in a mature human anagen hair follicle. (b) The upward movement of postmitotic cells differentiating to form the inner root sheath (two longest arrows), hair cuticle, hair cortex and hair medulla (shortest arrow). Modified, with permission, from Ref. 37.

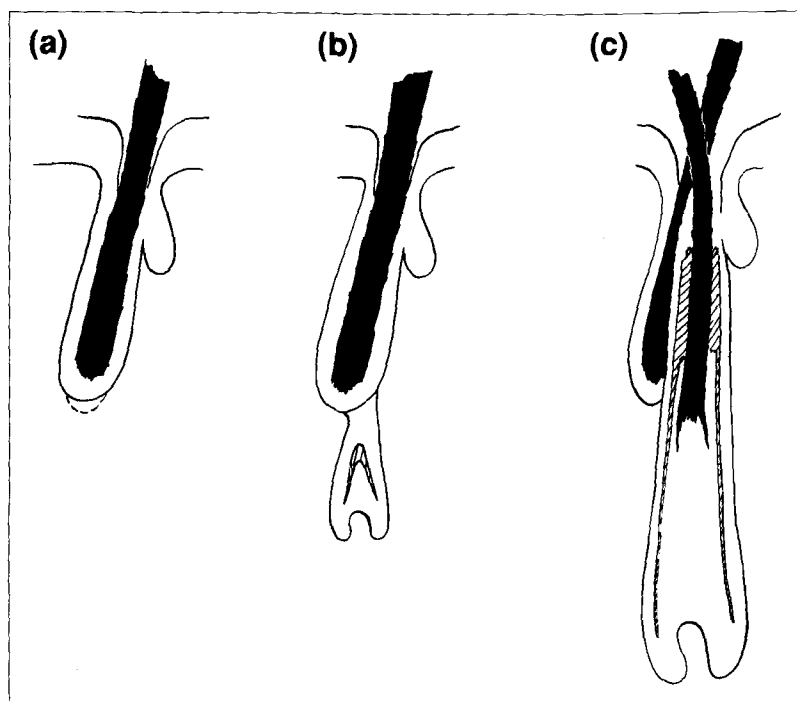


FIG 1

(a) A mouse telogen follicle with a fully keratinized hair at the base of a follicle that has shortened during anagen, and a cluster of dermal papilla cells (within dotted line) below the residual epithelial 'hair germ'. (b) A new anagen stage 4 growing from the base. (c) A fully grown anagen follicle with a new hair emerging beside the one that is in telogen. Reproduced, with permission, from Ref. 14.

other than the neuroendocrine ones involved with cycling in individual follicles.

Protein synthesis in the active hair follicle

In the sheep, over 100 distinct proteins synthesized by the hair cortex and cuticle cells produce the 'hard' keratin structure of wool fibres¹¹. These are related to but different from the proteins forming the 'soft' keratin of epidermis. The proteins of wool and other hairs are categorized as low-sulphur α -helical fibrillar proteins, and two nonfibrillar groups known as the high-sulphur and high-tyrosine proteins¹². The inner root sheath cells produce different fibrillar proteins, low in sulphur but rich in citrulline¹³. The inner root sheath gradually forms a rigid cylinder in the lower half of the follicle to act as a mould for the less rigid cells of the hair as they move together toward the skin surface. Yet another program of cytogenesis and citrulline-rich protein production forms a girder-like structure in the medulla of the hairs¹³.

It is difficult to imagine how all these synthetic processes are coordinated, resulting in the relatively uniform cylindrical scalp hair of a healthy human subject. It is even more difficult to imagine how, in each hair growth cycle of an animal such as the mouse, a primary follicle produces a long, thick straight hair with no medullated tip, while a later-formed follicle beside it produces its shorter, finer hair with a broad, medullated tip and a kinky, less medullated base. There must be a program in each follicle for assigning the daughter cells of hair matrix cell division to the different cell types and protein synthesis programs. The answers may lie in the hair matrix cells themselves, or in the dermal papilla.

There is more than meets the eye in a dermal papilla

Under the light microscope the dermal papilla in the first growth cycle appears as a collection of primitive mesenchymal cells in a loose extracellular matrix. Early histochemical studies showed strong reactions for mucopolysaccharides and alkaline phosphatase during anagen¹⁴. More recently, immunohistochemical and other *in situ* techniques have revealed other biochemical features that change during follicle development and hair cycles¹⁵. During development and throughout anagen phases the entire papilla is rich in basement membrane-type molecules such as heparan sulphate proteoglycan, and a chondroitin sulphate proteoglycan species distinct from that found in the general dermis. Fibronectin, another protein associated with basement membranes, is also prominent in the dermal papilla. The dermal papilla lacks the type I collagen that predominates in mature dermis, and even type III collagen is relatively sparse.

The basement membrane itself was thought to be continuous at the junction of the dermal papilla with the hair matrix at all times. However, electron microscopy revealed gaps in the basal lamina but only just before and during the differentiation of hair matrix cells into inner root sheath and hair cells¹⁶. Direct contacts between the plasma membranes of mesenchymal cell processes and epithelial cells were observed through these gaps. Thus there is a physical framework for possible cell-cell or cell-matrix interactions just at the time established experimentally for the second dermal message. Interestingly, the expression of the α -helical fibrillar keratin protein known as K14 was suppressed in hair matrix cells as long as they remained in close contact with dermal papilla cells¹⁷. Shortly afterwards, K14 was reported to be absent from all the cell types that differentiated from these hair matrix cells. In contrast, the other epithelial cells in the follicle, those of the outer root sheath, resembled the basal epidermal layer in that K14 was expressed at all times¹⁸. However, direct experimental evidence is needed to prove where any one of the biochemical or ultrastructural features reported here fits into signal transmission between the dermal papilla and hair matrix cells.

The search for regulator molecules

Because embryonic epidermis could be modulated by excess vitamin A in organ cultures of chick skin¹⁹, retinol and retinyl acetate have been added to embryonic mouse skin in similar cultures, to see whether developing hair follicles could also be diverted from keratin production to mucus secretion²⁰. Some pelage hair follicles, in the presence of excess retinoid, merely failed to form, while other follicles, which had already been initiated, developed normally for a few days but

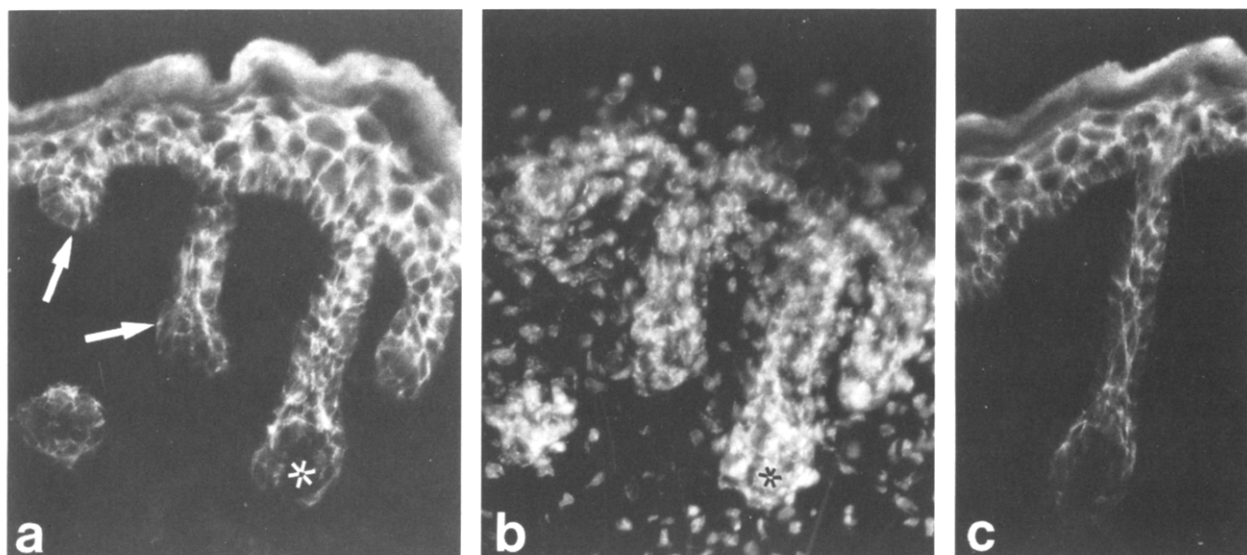


FIG 5

Localization of E-cadherin during the development of pelage hair follicles in mouse embryos with a rat monoclonal antibody (ECCD-2, a gift from M. Takeichi). Frozen sections of skin from the back were treated with this antibody followed by fluorescein isothiocyanate-labelled anti-rat IgG and examined by fluorescence microscopy. (a) Skin from a 17-day embryo showing E-cadherin expression on the cell membranes of all epidermal cells and all follicle epithelial cells. The same field is shown in (b), with fluorescent staining of all nuclei with Hoechst 33258. Comparison of (a) with (b) demonstrates that E-cadherin is absent from all the general dermal mesenchymal cells, the mesenchymal cells clustered below the epithelium of the early and late stage 2 follicles (arrows), and those within the dermal papilla of an early stage 3c follicle (asterisk in a, b). (c) Skin from an 18-day embryo, showing a more advanced stage 3c follicle with some E-cadherin on the surface of all epithelial cells. However, the intensity of the stain is greatly reduced on the innermost layer of the epithelial cells surrounding the dermal papilla. This is the future hair matrix, the area that, in vibrissa follicles, expresses more P-cadherin than E-cadherin³¹.

regressed at stage 3c, when they should be receiving the second dermal message. Vibrissa follicle development was also arrested around stage 3c, and the mesenchymal cell aggregations were dispersed. In addition, the vibrissa follicles developed lateral buds that grew into branching, mucus-secreting glands, as if responding to a new and different second dermal message, this time from the general dermis. Ultrastructural and other evidence in favour of mesenchymal-epithelial communication at the lateral walls of the follicles in the presence of retinoids is summarized elsewhere²¹. By growing lip skin in organ culture with retinoids, separating the dermis and recombining it with untreated epidermis in grafts to the chick chorioallantoic membrane, it was shown that the excess retinoids were acting on mesenchymal rather than epithelial structures to initiate the changes observed²².

It has been known for some years that all-*trans* retinoic acid (RA) is the active metabolite in most of the morphogenetic and cell differentiation programs affected by vitamin A. Three nuclear receptors for RA – RAR α , RAR β and RAR γ – have now been identified. *In situ* hybridization has detected transcription of the genes for each of these receptors in different parts of several developing mouse tissues, including vibrissa follicles. A cytoplasmic RA-binding protein (CRABP) was also located in the dermal cells forming a sheath around the lateral wall of the follicles, where it was thought to play a role in sequestering free retinoic acid^{23,24}. By applying the same *in situ* hybridization techniques to upper lip skin cultured for 48 hours with retinoic acid, it was shown that the RAR β gene was upregulated in the lateral dermal sheath of vibrissa follicles but not in

any other area²⁵. Since the recombination of this treated dermis with untreated epidermis grown in CM grafts led to the formation of mucous glands rather than hair follicles²², it was suggested that genes activated by RAR β could be responsible for the new dermal message.

Epidermal growth factor (EGF) was the first peptide growth factor reported to enhance cell proliferation, operating through a transmembrane glycoprotein receptor (EGFR) on basal epidermal cells and several other epithelial tissues. When large doses were given to Merino sheep by infusion, breaks and shedding of the fleece occurred²⁶. Hair follicle development in the mouse was also inhibited by EGF²⁶. In another study of rat embryos using autoradiography of ¹²⁵I-labelled EGF²⁷, it was noted that available EGF receptors were labelled throughout the basal layer of epidermis at 17 days, except for small patches above dermal mesenchymal cell condensations. These were identified as the stage 0 follicles at the time of the first dermal message. The epithelial cell patches remained unlabelled, as placode cells at stage 1, forming the base of hair plugs at stage 2 and as hair matrix cells at stages 4–8. Meanwhile, the outer root sheath cells and sebaceous gland cells became strongly labelled. The authors suggested that EGF or a related ligand could be involved in tissue induction by a mesenchymal tissue (i.e. the first dermal message?). It is worth noting that a related molecule, transforming growth factor α (TGF- α), binds to EGFR and also retards hair growth in mice²⁸.

In a study of another group of growth factors²⁹ it was found that transcripts of three members of the TGF- β superfamily – TGF- β_1 , TGF- β_2 and TGF- β_3 – showed different patterns of distribution in the various

epithelial and mesenchymal components of embryonic mouse vibrissa follicles, and that the patterns changed as the follicles developed. Another member of this superfamily, bone morphogenetic protein 2a (BMP-2a), was restricted to the epidermal placode in stage 1 follicles. The epidermal placode cells expressed this molecule throughout their migration as the base of the plug to become the hair and inner root sheath-producing group (Fig. 3) of the hair matrix cells in the late foetus, the newborn and the adult mouse. A further study³⁰ confirmed the pattern for BMP-2a, now known as BMP-2, and showed that a related factor, BMP-4, was restricted to the mesenchymal cell clusters of stage 0 and stage 1 follicles, but had disappeared by stage 2. The authors pointed out that BMP-4 could be acting as a signalling molecule from the mesenchymal cells to the overlying ectoderm. Would this be a strong candidate for the first dermal message?

In addition to retinoids and growth factors, some developmentally significant cell adhesion molecules have recently been located by immunohistochemistry in developing mouse vibrissa follicles³¹. E-cadherin, previously reported in mammalian epithelia (including developing epidermis), was located in all the epithelial cells of the stage 2 follicles (Fig. 5), while P-cadherin was largely restricted to the columnar cells next to the basement membrane in the deeper part of the follicle plug, that is, the future hair matrix cells. At a later stage (5 or 6) E-cadherin was located in the potentially proliferative cells of the hair matrix as well as some differentiating cells in the inner and outer root sheaths of developing mouse vibrissa follicles. P-cadherin was restricted to the proliferating hair matrix cells and the (proliferating) outer root sheath cells. In support of a significant role for P-cadherin in follicle morphogenesis, it was shown that anti-P-cadherin added to the culture medium of explanted lip skin caused the epithelial cells at the lateral margins of the hair plugs (at stages 2 and 3) to lose their columnar shape, and led to the dispersion of the mesenchymal cells that had been clustered around them to form the dermal sheath. Anti-E-cadherin by itself did not cause these changes, but it appeared to enhance the effects of anti-P-cadherin. Examination of the published photomicrographs suggests that the dermal papilla cells might also be dispersed, and in some cases the follicles had a rounded epithelial base when they should have been invaginated by a dermal papilla. These changes were remarkably similar to the earliest changes produced by excess retinoids in organ cultures of the same tissue²⁰.

Conclusion

We are only just beginning to understand how a hair follicle develops and functions. The combination of molecular with histological and histochemical approaches has been fruitful, especially when supplemented with experimental techniques such as organ culture and transplantation. The impressive list of known mutations affecting the hair of mice³² indicates another, rarely tapped resource for *in vivo* and *in vitro* experiments. [Preliminary investigations with *Naked* (*N*), *Tabby* (*Ta*), *fuzzy* (*fz*), *asebia* (*ab*) and others have been summarized^{33,34}.] Perhaps the hair

follicle, because of its small size and easy accessibility, could come to rival the vertebrate limb-bud³⁵ as a model for an intensive study of genetic regulation in morphogenesis.

Acknowledgements

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Upon exposure to foreign antigens, T lymphocytes can undergo a series of programmed biochemical changes that lead to the expression of the 'helper-inducer' or 'cytotoxic' T-cell phenotypes¹. This response, called T-lymphocyte activation, ensues from the interaction of an antigen bound to major histocompatibility complex (MHC) polypeptides located on an antigen-presenting cell (APC) with the antigen receptor of the T lymphocyte (Fig. 1). The ligand-binding domain of the T-cell receptor (TCR) for antigen is provided by the mutually exclusive $\alpha\beta$ and $\gamma\delta$ TCR heterodimers. These subunits are highly polymorphic and recognize unique antigens (antigen specificity) presented in the context of particular MHC molecules (MHC restriction). In addition to the ligand-binding subunits, the TCR contains five other chains collectively termed CD3- ζ (zeta), which participate in receptor assembly and/or the transduction of intracellular signals. The ζ chain is especially important for signal transduction: chimeric proteins consisting of T-cell surface molecules [such as CD8 or the α chain of the interleukin 2 (IL-2) receptor] and the intracellular portion of ζ are able to transduce biochemical signals, and trigger typical T-cell activation responses^{2,3}. As none of the TCR subunits, including ζ , possesses known catalytic properties, the mechanism by which this complex transduces intracellular signals has been the object of intense investigation.

The CD4 and CD8 T-cell surface antigens

In addition to the TCR, other surface molecules play important roles during T-cell activation. The best characterized of these 'accessory' activation molecules are the CD4 and CD8 antigens⁴ (Fig. 1). These two products of the immunoglobulin gene superfamily are monomorphic receptors for class II (CD4) and class I (CD8) MHC antigens. CD4 is a monomeric 55 kDa integral membrane glycoprotein. In contrast, CD8 exists as a disulfide-linked dimer or oligomer of two distinct transmembrane glycoproteins, the α (38 kDa) and β (30 kDa) subunits. Expression of the α subunit of CD8 is sufficient for recognition of class I MHC molecules and for providing an 'accessory' function during T-cell activation. The role of the β subunit is unknown. While CD4 is predominantly expressed on helper-inducer T lymphocytes, CD8 is primarily found on cytotoxic T cells. Immature thymocytes generally express both CD4 and CD8 on their surface.

Src-related protein tyrosine kinases and T-cell receptor signalling

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Upon antigen stimulation, the T-cell receptor for antigen transduces an intracellular protein tyrosine phosphorylation signal that is critical for subsequent T-lymphocyte activation. As the antigen receptor does not possess an intrinsic protein tyrosine kinase activity, the mechanism by which it regulates protein tyrosine phosphorylation is unconventional. Evidence is increasing that the Src-related protein tyrosine kinases p56^{lck} and p59^{fyn}, as well as the protein tyrosine phosphatase CD45, are involved in this process.

Transfection experiments in CD4⁺ CD8⁺ T cells reveal that expression of CD4 or CD8 significantly enhances responsiveness of T cells to low doses of antigen or suboptimal antigen stimulation whenever the TCR recognizes the same MHC molecule as CD4/CD8 (Refs 4, 5). Under these conditions, since the TCR and CD4/CD8 can presumably bind the same MHC molecule, CD4 and CD8 are thought to associate physically with the TCR during antigen stimulation. This has led to the view that CD4 and CD8 are conditional components ('co-receptors') of the TCR complex⁵. Part of the improved antigen responsiveness conferred by CD4 or CD8 expression results from the ability of these receptors to serve as adhesion molecules, enhancing physical interactions between the T cell and the APC⁴. In addition, evidence is accumulating that CD4 and CD8 can independently transduce biochemical signals during T-cell activation^{4–6}. This signalling function is dependent on the short cytoplasmic domains of CD4 and the α chain of CD8 and is likely to be mediated by the lymphocyte-specific protein tyrosine kinase p56^{lck} (Refs 6, 7; see below).

The lymphocyte-specific kinases p56^{lck} and p59^{fyn}

T lymphocytes express two major lymphocyte-specific protein tyrosine kinases: p56^{lck} and p59^{fyn}. p56^{lck} is a 56 kDa Src-related protein tyrosine kinase that is expressed exclusively in cells of lymphoid