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BEAUTY IS SKIN DEEP: THE FASCINATING BIOLOGY OF THE EPIDERMIS AND ITS APPENDAGES

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I. Introduction

The skin not only is the largest organ of the body, but it is also the organ that has received the greatest attention and fascination by man and by nature. From the colorful plumage of the peacock to the rainbow patterns of the parrotfish and the metallic green spots on the Raja Brooke butterfly, nature has clearly had more fun and fancy in creating the surface of organisms than she has in those tissues tucked beneath it. From the animal instincts of sexual attraction to the protective role of mimicry to the garish colorings designed to keep other organisms at a distance, the magnificence and cleverness displayed in creating body coverings has been not only beautiful but also valuable. All cultures have built upon the foundation of nature's imagery, adorning the body with paints and cosmetics, tattoos and jewelry. In general, this has been viewed in a positive fashion. But there has always been a tension between the body and the mind, resulting from the enormous attention given to the skin. As such, the skin has gotten a bit of a bad rap: "Beauty is only skin deep," we scoff, "It is our hearts, our minds and our souls which matter far more." And yet it is only the human who has been able to rationalize away the importance of skin in this manner, and, ironically, the mind itself that tempts us to tamper with our skin in ways that we so disdain. The fact is that for all organisms, the body surface, in all its splendid cloaks, is the source of survival and perpetuation.

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II. The Mammalian Epidermis and the Process of Terminal Differentiation

Since the time when I was a postdoctoral fellow under the supervision of Dr. Howard Green, then at the Massachusetts Institute of Technology, I began to follow my natural instincts of fascination with the skin to a deeper level, one that goes beneath the body surface and into the realm of skin biology. I have been interested in understanding the molecular mechanisms underlying growth, differentiation, and development in the mammalian epidermis and its appendages. The epidermis is the Saran Wrap of our body that protects against invasion by harmful microorganisms and that prevents essential bodily fluids from evaporating at our body surface (Fig. 1). The epidermis thus enables us to exist as terrestrial beings. The epidermis must also be able to withstand the various environmental and physical traumas to which we subject our body surface, survive when we wash our hands with soap, and reseal rapidly when we wound our skin. How does it accomplish these tasks?

The epidermis is the epitome of a self-renewing tissue: a single epidermal stem cell has sufficient proliferative capacity to provide enough new epidermis to cover the body surface (Rochat et al., 1994). The stem cells are tucked away in the innermost, basal layer of the epidermis, which is attached to a basement membrane of extracellular matrix. It has been postulated that periodically these cells divide asymmetrically to give rise to a daughter stem cell and a so-called transit-amplifying cell (Jones and Watt, 1993; Jones et al., 1995). The transit-amplifying cell then undergoes a limited number of divisions in the basal layer before it withdraws from the cell cycle, commits to differentiate terminally, detaches from the basement membrane, and begins to transit toward the skin surface (Barrandon and Green, 1987; for review, see Watt, 1998). As cells reach the body surface they are then sloughed, continually being replaced by inner cells moving outward. In this fashion the epidermis is normally under a constant state of dynamic equilibrium, replenishing itself every two weeks throughout life.

The process of terminal differentiation is morphologically and biochemically complex (Fig. 1). The transition from basal to first suprabasal layer is marked by major changes in gene expression, necessary for cells to discard their proliferative functions and adopt new protective roles. Genes involved in epidermal proliferation are shut off, while genes needed for

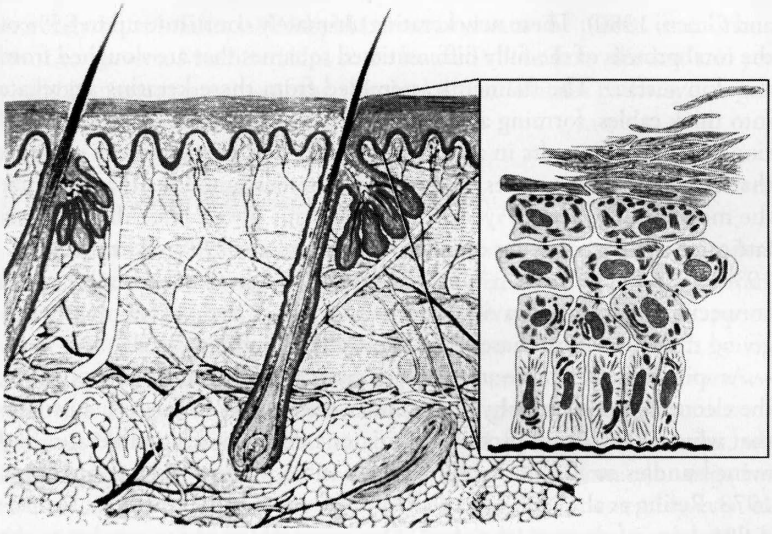


Fig. 1. The epidermis of mammalian skin. On the left is a schematic representing a cross-section of human skin. The areas in pink represent the epithelial components, including the epidermis, sebaceous gland, and hair follicle. The diagram at the right illustrates the stratified layers of the epidermis, depicting its four major steps in epidermal differentiation: (a) an innermost, basal layer of mitotically active cells, containing stem cells and transit amplifying cells; (b) three to six layers of spinous cells that are still transcriptionally active but are no longer dividing; these cells devote most of their translational machinery to expressing keratins; (c) one to three layers of granular cells that produce and transiently store the components of the lipid barrier; and (d) the stratum corneum, the dead, flattened cells held together by the seal of lipids secreted and organized at the end of the terminal differentiation process. (See color plates.)

providing the protective barrier function to the skin surface are turned on. Among the first changes are those in keratins, proteins that assemble into an extensive cytoskeletal network of 10-nm intermediate filaments, enabling epidermal cells to withstand the constant mechanical stresses that we subject our skin to daily (Fuchs, 1996; Fuchs and Weber, 1994). In the basal layer, keratin filaments composed of K5 and K14 are relatively scant and dispersed within the cell cytoplasm, imparting a modicum of strength to the cells while still enabling them to divide. As cells enter the suprabasal layers, they switch from expression of K5 and K14 to K1 and K10 (Fuchs

and Green, 1980). These new keratins ultimately constitute up to 85% of the total protein of the fully differentiated squames that are sloughed from the skin surface. The filaments assembled from these keratins aggregate into thick cables, forming a durable and dense network. The differentiation process also results in a marked increase and change in the proteins that comprise desmosomes, specialized adherens junctions that organize the metabolically active layers of the epidermis into a three-dimensional lattice of tightly adhering cells (Kowalczyk et al., 1999; Schmidt et al., 1994). Bundles of keratin filaments span the suprabasal cytoplasm and connect to the numerous desmosomal plaques at the plasma membrane, giving the cells within these layers the name of "spinous" cells.

As spinous cells move outward, they enter the granular layer, typified by the electron-dense keratohyalin granules composed of filaggrin, a protein that when released and processed initiates a dense packing of keratin filament bundles to form a nearly indestructible protein mass (Dale et al., 1978; Resing et al., 1993). In the final stages of metabolic activity, two additional sets of changes take place. The first is the finishing touches to the cornified envelope, a set of proteins that are sequentially synthesized and deposited beneath the plasma membrane of the differentiating epidermal keratinocytes (Christiano, 1997; Rice and Green, 1979). Loricrin, the last and major constituent of the envelope, is made in the granular layer, and after a brief storage period in small cytoplasmic granules, it relocalizes to the plasma membrane (Candi et al., 1995; Mehrel et al., 1990). As cells become permeabilized, an influx in calcium activates epidermal transglutaminases, which then through γ-glutamyl $\in$-lysine bonds, chemically cross-link the proteins into an indestructible envelope, which also interconnects the periphery of the keratin cytoskeleton (Rice and Green, 1979; Steinert and Marekov, 1995). Reaching the skin surface, these dead sacs or squames are packed with dense cytoskeletal forest, impenetrable by chemicals, detergents, or other environmental agents that could easily attack the cells of our internal organs.

While the keratin cytoskeleton is important for mechanical strength, it does not provide the epidermal barrier to keep microorganisms out and essential bodily fluids in. This is accomplished by the late-stage production and secretion of specialized lipids, creating a impenetrable seal to the skin surface (Downing, 1992; Elias, 1996; Harris et al., 1997; Wertz and Downing, 1982). During fetal development in humans, this barrier develops late in pregnancy, at approximately 8.5 months of gestation. Pre-

maturely born infants lack a lipid barrier and must be placed in an incubator to survive. Much remains to be learned about how this last and yet vitally important process of the terminal differentiation pathway is regulated in the skin.

III. Genetic Disorders of Epidermal Differentiation

In recent years, major advances have been made not only in our understanding of the structural changes that take place during terminal differentiation in the epidermis, but also in our understanding of what happens when this process is defective. My own laboratory's research began by focusing on the keratins. After studying the process of 10-nm filament assembly, we began to explore whether there might be genetic disorders of keratin. Through our studies on the K5 and K14 pair of keratins, we learned that most mutations in keratins behave in a dominant negative fashion, that is to say that they perturb 10-nm filament assembly even in the presence of their wild-type partner keratin (Albers and Fuchs, 1987, 1989; Coulombe et al., 1990; Letai et al., 1992; Wilson et al., 1992). Based on these findings, we predicted that the majority of keratin disorders should display an autosomal dominant pattern of inheritance, and we learned which regions of the proteins were most critical to the assembly process.

But what human genetic diseases were likely to be keratin disorders? In order to address this question, we used transgenic mouse technology to target the expression of a gene encoding a defective keratin to the skin (Coulombe et al., 1991a; Vassar et al., 1991). In the first set of experiments, transgenic mice expressing a mutant human keratin 14 gene displayed the clinical and pathological features of epidermolysis bullosa simplex (EBS), a blistering human skin disorder involving cytolysis or degeneration of the dividing, that is, basal layer, of the epidermis (Vassar et al., 1991) (Table I). Mutant K14 proteins that most severely disrupted keratin filament formation displayed more severe skin blistering (Coulombe et al., 1991a). In the most severe form of the disorder (Dowling Meara EBS), aggregates of keratin were present in the basal, but not the suprabasal layers of the epidermis.

The transgenic mice gave us the clues we needed to focus on the human form of this disease. We obtained skin biopsies from patient volunteers who suffered from EBS. Taking advantage of the remarkable self-renewing

TABLE I. CHARACTERISTICS OF EPIDERMOLYSIS BULLOSA SIMPLEX

Feature	Dowling-Meara	Koebner	Weber-Cockayne
Autosomal dominant	+	+	+
Skin blistering	Entire body	Body	Hands/feet
Basal cell cytolysis	+	+	+
Discernable abnormalities in basal keratin network	+	+	±
Keratin clumping in basal layer	+	−	−
Oral involvement	+	−	−

capacity of the epidermis, we cultured epidermal cells from patient volunteers who suffered from EBS. Upon cloning and sequencing the K14 and K5 genes from these keratinocytes, it was soon discovered that humans with EBS have point mutations in their K14 or K5 genes (Bonifas et al., 1991; Coulombe et al., 1991b; Lane et al., 1992), and that these defects reside at chromosomes 17q12-21 and 12q11-12, that is, at the loci of the type I and type II keratin gene clusters, respectively (Bonifas et al., 1991; Chan et al., 1993, 1994; Rosenberg et al., 1988, 1991). These mutations resided in regions that were known to perturb keratin filament assembly.

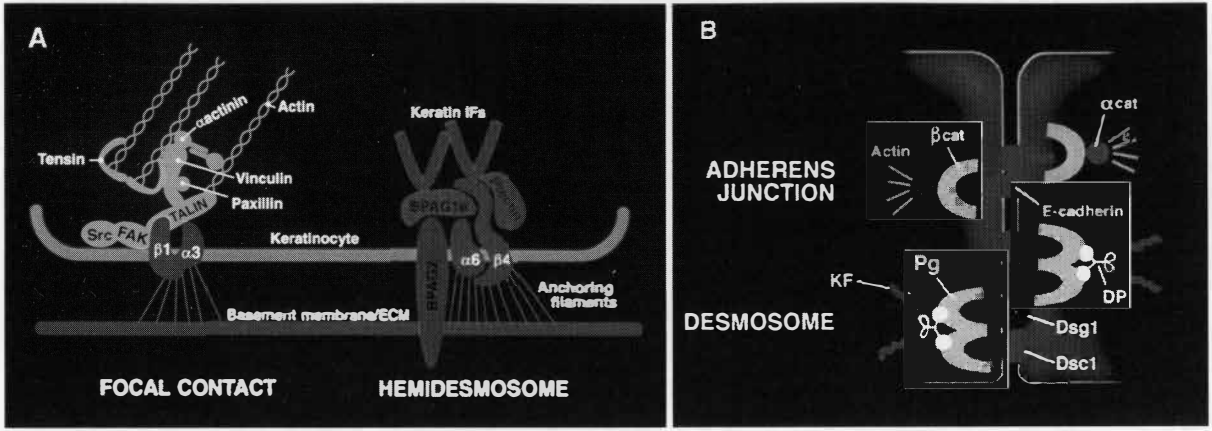
The work represented the first example where the genetic basis of a human disease had been correctly predicted from transgenic mouse technology. It also revealed that the function of the keratin network is to impart mechanical integrity to cells, without which the cells become fragile and prone to rupturing upon mechanical stress. It further led to the correct prediction that a related disorder, epidermolytic hyperkeratosis (EH) would be a genetic disorder involving mutations in K1 and K10: in EH patients the basal cells are healthy, but the suprabasal cells of the epidermis display cytolysis and perturbations in their keratin networks (Chang et al., 1992; Chipev et al., 1992; Rothnagel et al., 1992).

Once the paradigm for a keratin disorder was established, elucidating the genetic disorders of keratin became a straightforward process. To date, the growing list of keratin disorders has surpassed 10 (Table II) (for review, see Fuchs and Cleveland, 1998). This work also led to the realization that there are likely to be degenerative disorders involving other members of the intermediate filament superfamily. Indeed, aberrations in neurofilament gene

TABLE II. DISORDERS OF INTERMEDIATE FILAMENTS AND THEIR CYTOSKELETAL NETWORKS

Disorder	Cells involved	Species	Genes mutated
Epidermolysis bullosa simplex (EBS) Dowling-Meara EBS Koebner EBS Weber-Cockayne EBS	Basal epidermal (bas. epi.)	Mouse/human	K5, K14
EBS w/mottled pigmentation	Bas. epi.	Human	K5
EBS w/muscular dystrophy	Bas. epi./muscle	Human	Plectin
EBS w/sensory neuron degeneration	Bas. epi./DRG*	Mouse	BPAG1
Junctional epidermolysis bullosa	Epi./dermal junction	Mouse/human	α6β4 Laminin 5
Epidermolytic hyperkeratosis (EH)	Suprabasal epi.	Mouse/human	K1, K10
Ichthyosis bullosa of Siemens (mild EH)	Upper suprabasal	Human	K10, K2e
Epidermal nevi/EH type (mosaic EH)	Suprabasal epi./mosaic	Human	K1, K10
Epidermolytic palmoplantar keratoderma	Suprabasal palmoplantar	Human	K9
Pachyonychia congenita	Nails, hair, Epidermis near Follicle openings	Mouse/human	K6, K16, K17
White sponge nevus	Oral epithelia Esophagus	Human	K4, K13
Monilethrix	Hair	Human	Ha/Hb Keratins
Chronic hepatitis	Liver	Mouse	K18
Motor neuron disease	Motor neurons	Mouse	NFs
Generalized myopathy	Muscle	Mouse/human	Desmin

*DRG, dorsal root ganglia


A
αactinin
Actin
Keratin IFs
Tensin
Vinculin
Paxillin
TALIN
Src FAK
β1 α3
Keratinocyte
BPAG1e
BPAG2
α6 β4
Anchoring filaments
Basement membrane/ECM
FOCAL CONTACT
HEMIDESMOSOME
B
ADHERENS JUNCTION
Actin
βcat
αcat
E-cadherin
DP
KF
Pg
DESMOSOME
Dsg1
Dsc1

expression can lead to motor neuron disease in mice (Cote et al., 1993; Xu et al., 1993), and defects in the desmin gene cause generalized muscle degeneration in mice and in humans (Goldfarb et al., 1998; Li et al., 1996; Milner et al., 1996; Munoz-Marmol et al., 1998).

Epidermal cells are not simply bags of keratin filaments. Rather, keratins spin an intricate web of filaments that stretch out across a cell, extending from the nuclear envelope in the center of the cell to the desmosomes and hemidesmosomes at the cell periphery. As in all stratified squamous epithelia, the basal layer of the epidermis contains numerous hemidesmosomes, which are electron-dense membrane plaques located at the base where the epithelium attaches to the basement membrane (Fig. 2A). Filaments composed of laminin 5 anchor hemidesmosomes to the underlying basement membrane. At the core of the hemidesmosome is a cluster of $\alpha6\beta4$ integrins, unusual in that they attach to the keratin filament network rather than the actin microfilaments (for review, see Garrod, 1993; Jones et al., 1998; Schmidt et al., 1994). Three additional proteins, BPAG1-e,

Fig. 2. Models of cell-substratum and cell-cell junctions in the epidermis. A. Cell-substratum junctions. Focal contacts and hemidesmosomes are characteristic of all stratified squamous epithelia. Focal contacts form around clusters of $\alpha3\beta1$ integrins, whose function it is to organize the basement membrane of extracellular matrix to which the epidermis adheres (DiPersio et al., 1997; Hodivala-Dilke et al., 1998). These junctions attach to the actin cytoskeleton and are hence likely to be important for epidermal migration and movement. In contrast, hemidesmosomes attach to the intermediate filament network. At their core are clusters of $\alpha6\beta4$ integrin heterodimers. They are more robust structures, visible at the ultrastructural level as electron dense membrane plaques. Both $\alpha3\beta1$ and $\alpha6\beta4$ use laminin 5 as their ligand, but the lion's share of cell-substratum adhesion to the basement membrane is performed by hemidesmosomes (Dowling et al., 1996; Georges-LaBouesse et al., 1996; Van der Neut et al., 1996). **B.** Cell-cell adhesion. Adherens junctions in epidermal cells are made through calcium-activated, homotypic intercellular interactions of E-cadherins. On the cytoplasmic side, these transmembrane domain proteins associate with β-catenin, which in turn associates with α-catenin and the actin cytoskeleton (for review, see Garrod, 1993; Kowalczyk et al., 1999; Schmidt et al., 1994). In contrast, desmosomes associate with the keratin filament cytoskeleton. At the core of these structures are desmogleins and desmocollins, members of the cadherin superfamily. The cytoplasmic domains of these proteins associate with plakoglobin and plakophilin 1, members of the β-catenin superfamily. Desmosomal cadherins, plakoglobin, and plakophilin 1 all have the ability biochemically to associate with desmoplakin, a homodimeric protein which through its amino terminal domain clusters the other desmosomal components, and through its carboxyl terminal domain anchors the intermediate filament cytoskeleton (Troyanovsky et al., 1993; Kouklis et al., 1994; Kowalczyk et al., 1996; Smith and Fuchs, 1998; Stappenbeck et al., 1993). (See color plates.)

BPAG2, and plectin, impart to the hemidesmosome its distinctive structure. BPAG1-e is a protein that resides at the inner plate of the hemidesmosome where the keratin filaments seem to thread through the inner surface; BPAG2 is a transmembrane protein with an extracellular domain similar in sequence to secreted collagens. These unusual BPAG proteins receive their name from the fact that patients with the autoimmune disease bullous pemphigoid produce autoantisera against these proteins (for review, see Stanley, 1993). Plectin is a protein that shares sequence similarities with BPAG1-e, but it differs in that it localizes not only to hemidesmosomes but also to the keratin cytoskeleton (Wiche, 1998).

What is the function of the hemidesmosome? Recently, we used gene targeting technology to ablate β4 integrin in mice (Dowling et al., 1996; see also Georges-Labouesse, et al., 1996; van der Neut et al., 1996). In the absence of β4, α6 is unstable leading to a complete loss of hemidesmosomes in these animals. The mice develop clinical signs of a devastating human blistering disorder, referred to as junctional epidermolysis bullosa. Patients with this disorder have been found to have premature stop codons or small internal deletions in either their laminin 5 chains, β4, or α6 (Vidal et al., 1995; for review, see Christiano and Uitto, 1996; Pulkkinen and Uitto, 1998). In our null mice, the complete loss of hemidesmosomes leads to a drastic weakening of cell-substratum contacts (Dowling et al., 1996). These functions seem to be distinct from those of α3β1 integrins, which even though present in basal epidermal cells, do not seem able to compensate for the loss of α6β4. The α3β1 integrin, which attaches to the actin cytoskeleton, appears to play a role in organizing the extracellular matrix of the basement membrane (DiPersio et al., 1997).

What is the function of the attachment of keratin filaments to the hemidesmosome? To answer this question, we used gene targeting to ablate the BPAG1 gene in mice (Guo et al., 1995). Removing the coiled-coil BPAG1-e protein from the hemidesmosome severs the connection between the hemidesmosome and its keratin filament network, leading to a narrow zone of mechanical fragility just above the base of these epidermal cells (Guo et al., 1995). While the phenotype is somewhat more complex due to the plectin's ability to associate with both the actin and keratin cytoskeletons, ablation of plectin in mice also results in basal cell fragility (Andra et al., 1997). Thus, this attachment of filaments to the base of the basal layer appears to protect these cells against the sheer forces exerted whenever our skin is rubbed.

Keratin filaments are also attached to desmosomes, specialized types of cadherin-mediated cell-to-cell junctions that interconnect all cells within the epidermis (Fig. 2B; for review, see Kowalczyk et al., 1999; Schmidt et al., 1994). While epidermal junctions composed of E-cadherins link to the actin cytoskeleton through β- and α-catenins, those composed of desmosomal cadherins link to the keratin cytoskeleton through three potential candidate proteins—desmoplakin, plakoglobin, and plakophilins. Based upon recent genetic studies, all three of these proteins appear to be involved not only in connecting the keratin filaments to desmosomes, but also in desmosome assembly and/or stabilization. This is perhaps best exemplified by the fact that genetic defects in plakophilin 1 cause congenital ectodermal dysplasia, an autosomal recessive human genetic disorder involving suprabasal epidermal degeneration and a loss of cell-cell adhesion (McGrath et al., 1997).

In mice, ablation of the plakoglobin and desmoplakin genes results in embryonic lethality. Plakoglobin null embryos die of massive defects in the developing heart muscle, whose cells lack desmosomes and intercellular adhesion (Bierkamp et al., 1996; Ruiz et al., 1996). Desmoplakin null embryos barely make it past implantation, unable to form an egg cylinder, the first major step in developing the shape of the animal (Gallicano et al., 1998). In contrast to E-cadherin junctions which are present in the two cell mouse embryo and which are necessary for blastocoel cavity formation (Ohsugi et al., 1997; Riethmacher et al., 1995; Torres et al., 1997), desmosomes first appear in the outer (trophectoderm) layer of the blastocyst and are not found in the inner cells of the developing embryo until gastrulation (Jackson et al., 1981). These findings, coupled with their prominent ultrastructure, imply that desmosomes are required for processes requiring more robust intercellular adhesion.

Overall, the phenotypes arising from perturbations in hemidesmosomes and desmosomes tells us that the function of these structures is in adherence, in one case to the cell substratum and in the other to intercellular connections. In contrast, the connections of these junctions to the keratin filament cytoskeleton impart mechanical strength by providing a filamentous framework within the epidermal cells.

What do we know about the functions of the proteins involved in the late stages of terminal differentiation and about their relation to human disease? We know that patients who lack transglutaminase 1, the major epidermal transglutaminase, do not produce proper cornified envelopes

and suffer from lamellar ichthyosis, a disorder typified by generalized scaling, hyperkeratosis, and defective barrier function (Candi et al., 1998; Huber et al., 1995; Matsuki et al., 1998; Russell, et al., 1995). Patients with genetic defects in loricrin suffer from Vohwinkel's syndrome, involving thickening and scaling of the palms and foot soles and constrictions of the fingers (Maestrini et al., 1996). Patients with genetic defects in steroid sulfatase display the characteristics of X-linked ichthyosis, where the skin surface adopts an appearance of fish scales, and the barrier function of the epidermis is defective (Shapiro et al., 1978; Webster et al., 1978; Yen et al.,1987). As we learn more about the molecular pathways involved in late-stage epidermal differentiation, additional insights will undoubtedly be gained regarding the genetic bases of disorders of the epidermal barrier function.

IV. The Hair Follicle: The Major Appendage of the Epidermis

Far less is known about the structure of the hair follicle than the epidermis. A major reason for this is due to the complexity of the follicle. Early in development, the epithelium begins as the ectoderm, a single layer of cells that are pluripotent and can give rise to either follicle or epidermis. The decision-making process depends upon whether the ectoderm makes contact with a condensate of specialized mesenchyme, called the dermal papillae. A mesenchymal signal cues an ectodermal cell to proliferate and grow downward to form a hair germ (Hardy, 1992). An ectodermal cue then engulfs the dermal papillae and prompts these epithelial cells to differentiate further to develop into a hair follicle, forming a compartment of stem cells, a sebaceous gland, and a hair shaft surrounded by an outer and inner root sheath (Fig. 3).

The stem cell compartment is thought to reside in the bulge, just below the sebaceous gland. These cells, the dermal papillae and the sebaceous gland are permanent fixtures of the follicle. In contrast, the lower two-thirds of the adult hair follicle cycles undergoing periods of growth (anagen), rest (catagen), and regression (telogen). Growth appears to be dependent upon periodic stimulation of a stem cell by the dermal papilla cells. The stem cell is subsequently converted to matrix cells, the transit-amplifying cells of the hair follicle. These cells proliferate and grow downward, maintaining contact with the papilla. As cells lose contact with

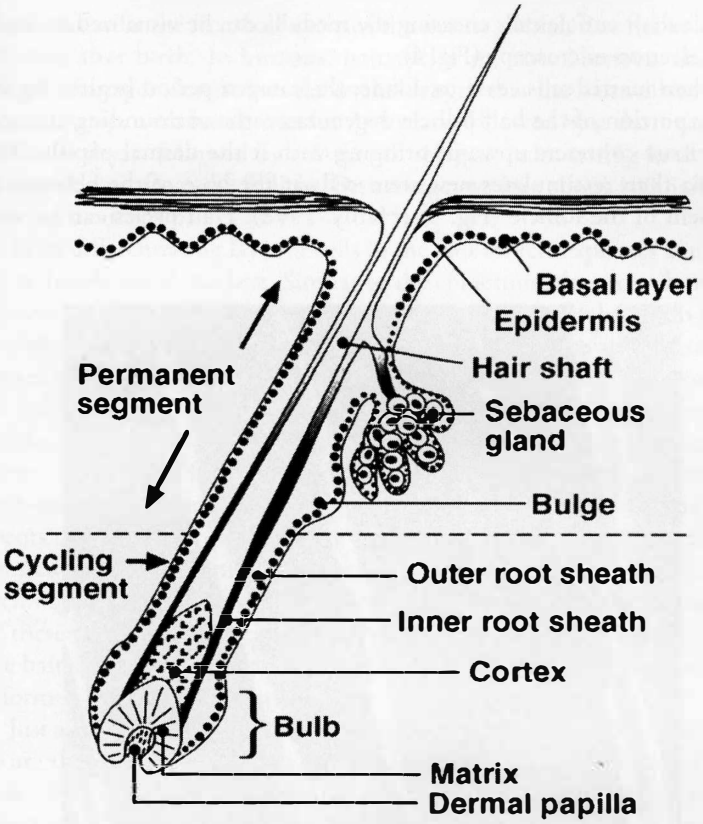


Fig. 3. The hair follicle. The sebaceous gland, upper outer root sheath (including the bulge), and mesenchymally derived dermal papilla are established once, during embryonic follicle morphogenesis. The lower portion cycles postnatally by a process involving transient stimulation by the dermal papillae of follicle stem cells, thought to reside in the bulge. Matrix cells are transit amplifying cells that differentiate upward in concentric rings of cells, ultimately producing the hair shaft and its inner sheath.

mesenchyme, they choose one of six different programs of terminal differentiation, creating concentric rings of differentiated cell types, which from inside to out include: medulla or hair shaft, shaft cuticle, inner root sheath cuticle, Henle and Huxley inner root sheath layers, and outer root sheath. Each hair that breaks the skin surface is composed of dead cells. The layer

of hair shaft cuticle cells encasing the medulla can be visualized by scanning electron microscopy (Fig. 4).

When matrix cells cease to divide, the catagen period begins. As the lower portion of the hair follicle degenerates, the surrounding connective tissue contracts upward, bringing with it the dermal papilla. The papilla then restimulates new stem cells at the base of the permanent segment of the follicle (Fig. 3) (Hardy, 1992). Hair cycles can be syn-

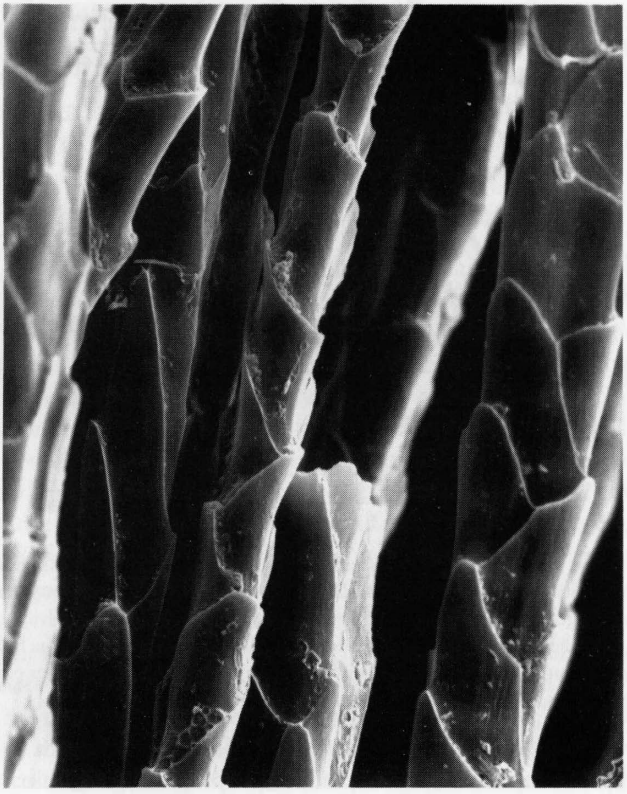


Fig. 4. A close-up view of mouse hair. Scanning electron micrograph of mouse hair, illustrating the cellular nature of the hair shaft. The visible outer layer of the shaft is the shaft cuticle. Inside are the medulla cells. All of the cells of the hair shaft are dead skeletons, composed largely of large cables of hair keratin filaments. Magnification is 12,500×.

chronous, as is the first hair cycle of the mouse, which finishes at about 18 days after birth. In humans, hair cycles are asynchronous, and the length of anagen determines the overall length that the hair will achieve. For the scalp, each hair grows for 3–5 years, followed by a short catagen (about 3 days), followed by a 3-month telogen, culminating in the loss of the old hair from the follicle. On average, we lose about 100 hairs per day from our scalp.

Each differentiating layer of cells in the hair follicle expresses a unique set of biochemical markers. Similar to the epidermis, the most abundant proteins of the hair follicle are the keratins. The Ha and Hb keratin genes encode a set of keratins that are only expressed in the differentiating cortex cells that give rise to the hair shaft (Powell et al., 1991). In contrast to epidermal keratins, the hair-specific keratins are rich in cysteine residues, many of which protrude along the surface of the keratin filaments (Powell and Rogers, 1997; Rogers et al., 1997, 1998). Additional hair-specific, cysteine-rich proteins interact with the hair keratin filaments to produce larger and more rigid bundles of filaments than are expressed in the epidermis (Fietz et al., 1993; Fratini et al., 1993). The technique involved in a permanent wave at the hair salon takes advantage of these facts: after reducing the disulfide bonds in the cytoskeletons of the hair shaft cells, the shaft is molded into a curl and the bonds are then reformed upon "neutralization."

Just as there are genetic disorders of keratins that involve the epidermis, so are there genetic disorders of the hair keratins and its associated proteins. Monilethrix is a degenerative disorder involving brittle hair in humans, and not surprisingly it was recently shown to be a disorder involving the Ha and Hb keratins (Winter et al., 1997a,b). Similarly, transgenic mice displaying perturbations in their hair keratin networks display signs of hair brittleness, leading to balding (Powell and Rogers, 1990). Desmosomal gene defects can also give rise to hair abnormalities: expression of a mutant Dsg3 gene in mice causes alterations in the hair coat, and a lack of Dsg3 in mice is the cause of a spontaneous recessive mouse mutant, given the name "*balding*" (Koch et al., 1997). Desmoglein 3 is one of the transmembrane cadherin proteins that is at the core of the desmosomes in the basal and suprabasal layers of the epidermis and also in the outer root sheath of the hair follicle (Koch et al., 1998; Kurzen et al., 1998). Presumably, the expression of other desmosomal cadherins in the epidermis compensates for the loss of Dsg3 in this tissue.

V. Development and Transcriptional Regulation in the Epidermis: What Controls Keratinocyte-Specific and Differential Programming of Gene Expression?

While much is known about the patterns of expression of structural genes in the epidermis and its appendages, much less is known about how these patterns are established during development and how the programs of terminal differentiation are orchestrated at the transcriptional level. Epidermal-specific gene expression has been more extensively studied than hair-specific transcription, predominantly because of the ability to culture and transfect epidermal keratinocytes *in vitro* and to simulate terminal differentiation by monitoring the levels of calcium in the culture medium. In addition, for both basally and suprabasally expressed genes, promoter/enhancer segments have been identified that can target epidermal-specific and differentiation-specific expression of reporter genes in transgenic animals.

As the study of keratinocyte-specific gene expression progressed, the binding sites for three major classes of transcription factors surfaced: AP2, Sp1, and AP1 (for review, see Byrne, 1997). Where tested, these sites were shown to be functionally important for conferring cell type-specific gene expression in tissue culture, and they bound factors that were more abundant in keratinocytes than many other cell types (see, e.g., Byrne et al., 1994; Casatorres et al., 1994; DiSepio et al., 1995; Jang et al., 1996; LaPres and Hudson, 1996; Leask et al., 1990, 1991; Snape et al., 1990). In addition, a number of mRNAs encoding AP2 or AP1 family members, including AP2α, AP2γ and c-fos, are expressed quite prominently in the epidermis and its hair follicles (Byrne et al., 1994; Smeyne et al., 1992). Additionally, a number of the transcription factor genes purported to control basal epidermal-specific gene expression are expressed in early embryonic development just prior to the expression of K5 and K14, and at a time when the ectoderm is still a single-layered epithelium (Byrne et al., 1994). Despite these promising correlations, gene knockout studies have yet to reveal an unequivocal requirement for any specific AP2, Sp1, or AP1 factor in epidermal-specific gene expression.

Two rather curious and yet nearly universal findings have emerged regarding keratinocyte-specific promoters: (1) they contain functional Sp1, AP2, and AP1 binding sites in the apparent absence of any family members that seem to be keratinocyte-specific, and (2) these sites occur in epidermal promoters regardless of the stage in differentiation at which these

genes are expressed. The first of these findings raises the question as to whether keratinocyte-specific transcription may be achieved through the combinatorial action of different members of these transcription factor families. The second suggests the possibility that flanking sequences may influence the degree to which a specific family member can bind and/or activate the gene. In addition, there could be keratinocyte-restricted or preferred co-factors that might interact with and influence the activity of these family members. A more complete understanding of the role of these and other factors in epidermal-specific and differentiation-specific gene expression must await more comprehensive studies exploring in detail the activities of multiple epidermal promoters in cultured epidermal cells and in transgenic mice.

VI. Gene Expression and Morphogenesis in the Hair Follicle: Balancing Proliferation and Differentiation

Elucidating transcriptional regulation in the hair follicle has been hampered by the lack of a culture system capable of inducing hair-specific gene expression *in vitro* and by the complexity of differentiation in the hair follicle. Like the epidermally expressed genes, hair keratin genes often have AP2, Sp1, and AP1 binding motifs in their promoters (Dunn et al., 1998; Powell et al., 1991, 1992). Interestingly, this group of hair-specific promoters also harbors sequence motifs for and binds to the Lef1/Tcf family of DNA binding proteins (Dunn et al., 1998; Zhou et al., 1995). First found as factors involved in lymphoid-specific gene expression (Travis et al., 1991; Waterman et al., 1991), Lef1 was subsequently shown to be expressed in developing hair follicles, where its mRNAs first appear in the ectodermal placodes that are the sites for follicle morphogenesis (Zhou et al., 1995). *In vitro* organogenesis studies on developing hair follicles and teeth suggest that bone morphogenic protein-4 (BMP-4) may be responsible for inducing the expression of Lef1 in this fashion (Keranen et al., 1998; Kratchowil et al., 1996; see also Vainio et al., 1993).

In the postnatal hair follicle, Lef1 mRNA expression is restricted to the matrix cells of the postnatal follicle (Gat et al., 1998; Zhou et al., 1995). This was surprising since these cells are the precursor cells to the hair keratin-expressing cells. Taken together, the inverse correlation between Lef1 expression and hair-specific gene expression in the hair follicle suggest that

Lef1 might act as an inhibitor for this set of genes. Consistent with this notion is the finding that when the Lef1/Tcf site is mutated in a hair keratin promoter, hair-specific gene expression still occurs (Byrne and Fuchs, unpublished data; Dunn et al., 1998).

The expression pattern of Lef1 suggests that this factor may play a positive role earlier in hair follicle morphogenesis. In support of this notion is the Lef1 knockout mouse, which is notably devoid of whiskers and has severe impairment of body coat hairs (van Genderen et al., 1994). In addition, overexpression of Lef1 driven from the K14 promoter resulted in perturbations in the hair coat and the appearance of hairs in inappropriate places (Zhou et al., 1995).

The mysterious role of Lef1 as a player in hair follicle formation and hair-specific gene expression began to unravel when it was soon recognized that Lef1 was the missing factor in the Wnt/wingless signal transduction pathway, implicated in many developmental processes (Willert and Nusse, 1998). Beta-catenin typically functions only in cadherin-mediated cell-cell adhesion, and any excess cytoplasmic β-catenin is rapidly degraded through a phosphorylation-dependent, ubiquitin-mediated process (Aberle et al., 1997; Orford et al., 1997). Upon a Wnt signal, the GSK3 kinase is inhibited, resulting in the stabilization of β-catenin and its interaction with Lef1/Tcf family members. Together, the two act as a transcription factor with Lef1/Tcf contributing the DNA binding domain and β-catenin supplying the transactivating domain (van de Wetering et al., 1997).

To test the hypothesis that Lef1 might function with β-catenin in hair follicle morphogenesis, Gat et al. (1998) expressed a constitutively stable form of β-catenin under the control of the K14 promoter. In this case, evidence of *de novo* hair follicle morphogenesis was apparent in postnatal mouse skin, giving the mice a very furry appearance (Fig. 5). New interfollicular invaginations began to appear at about d18. Induced in both interfollicular and outer root sheath cells of the transgenic animals, these *de novo* hair germs displayed nuclear Lef1, which by some mechanism, presently not clear, was either induced or stabilized in the β-catenin-activated cells. The induction of new hair follicle production took place only in haired skin regions and seemed coincident with the initiation of the first postnatal hair cycle. This has led us to surmise that perhaps the as yet unidentified signal(s) transmitted by dermal papillae to instruct existing follicles to begin the next hair cycle might also cue to the interfollicular

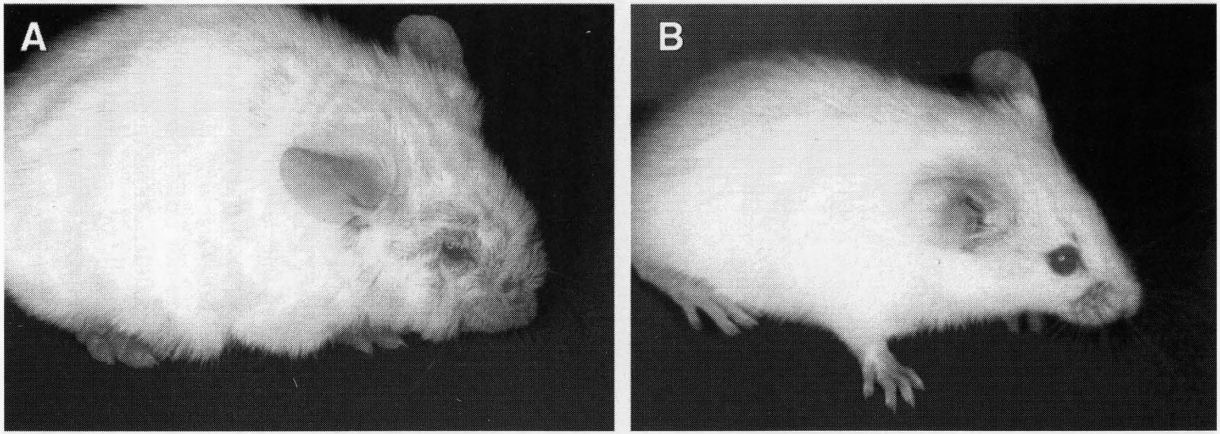


Fig. 5. Engineering a furry mouse. **A.** Transgenic mouse generated by using a basal epidermal keratin 14 (K14) promoter to drive the expression of a stabilized form of β-catenin (Gat et al., 1998). The engineered form of β-catenin lacks the normal regulatory machinery of the protein which would otherwise target any excess cytoplasmic β-catenin for phosphorylation and ubiquitin-mediated degradation (Aberle et al., 1996). **B.** Control littermate.

transgenic β-catenin expressing epidermis to initiate the formation of brand new follicles.

Taken together, these recent findings suggest that stabilization of β-catenin in postnatal epidermis results in its conversion to an embryonic state, competent to form either epidermis or hair follicles. In other words, the ability to stabilize β-catenin may be a key characteristic of pluripotent ectodermal stem cells, and the simultaneous induction of Lef1 expression by a dermal cue may then commit a stem cell to a hair follicle cell fate. The best candidate for a molecule to stabilize β-catenin is a Wnt family member, and in this regard, Wnt 10b (St. Jacques et al., 1998) and Wnt 7a (Chuong et al., 1996) have been shown to be expressed in vertebrate skin. We predict that a characteristic of pluripotent stem cells will be Wnt-responsiveness, presumably through expression of Wnt receptors.

Our studies further predict that the activation of β-catenin-Lef1 as a transcription factor is key in converting a follicle stem cell into a transit-amplifying matrix cell. A clue as to how this might happen stems from the recent studies of He et al. (1998), who discovered that c-myc is a downstream target gene for β-catenin/Tcf4 activation in intestinal epithelium. N-myc has been shown to be expressed specifically in hair matrix cells (Sutton et al., 1991), and either N-myc and/or c-myc activation could readily account for the transient induction of matrix cell proliferation that occurs during hair morphogenesis in development, and during anagen in cycling follicles. Intriguingly, members of the myc family have also been implicated in balancing epidermal proliferation and differentiation (Hurlin et al., 1995), suggesting the possibility that β-catenin stabilization could also be important for converting an epidermal stem cell to a transit-amplifying cell in the basal layer of the epidermis. If so, we surmise that another Lef/Tcf family member and/or another β-catenin co-factor might be involved, since the only prominent Lef1 mRNA detected in postnatal skin is in the matrix cells of the hair follicle (Gat et al., 1998; Zhou et al., 1995). The basal layer ceases to express high levels of Lef1 mRNAs after the final sets of hair follicles have been established.

Hair follicle formation requires not only matrix cell proliferation, but also remodeling of the intercellular connections within the developing follicle. This phenomenon could be accounted for if recruitment of β-catenin as a transcription co-factor results in concomitant weakening of those intercellular junctions mediated by β-catenin. A model

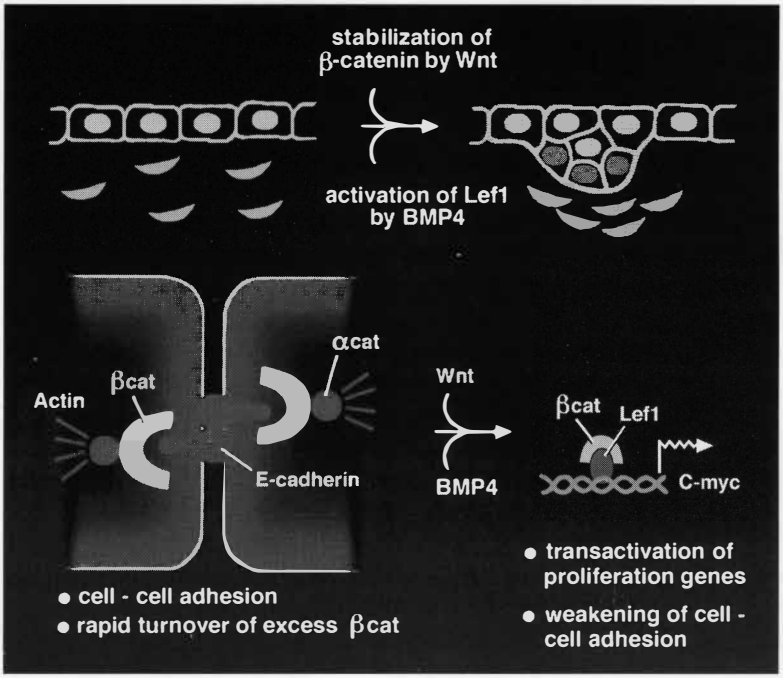


Fig. 6. Model depicting β-catenin's roles in hair follicle morphogenesis. Skin epithelium begins as a single layer of pluripotent cells, able to choose between an epidermal or hair follicle cell fate. A mesenchymal signal(s) cues the ectoderm to initiate a hair follicle. An ectodermal signal then prompts epithelial expansion and the organization and engulfment of a specialized dermal condensate, referred to as the dermal papilla. Additional mesenchymal cues complete the formation of the hair follicle (for review, see Hardy, 1992). The finding that β-catenin stabilization can cause an adult epidermal cell to act as if it were embryonic ectoderm suggests that a Wnt signal is an important external cue to the ectoderm (Gat et al., 1998). Since Lef1 expression occurs in the ectodermal placodes preceding dermal papilla formation (Zhou et al., 1995), induction of Lef1 by mesenchymal BMP-4 is likely to be a second key signal (Kratochwil et al., 1996). Upon stabilization of β-catenin and accumulation of Lef-1, the ectodermal signal is now generated. This results in the transcriptional activation of downstream targets, one of which is myc, known to play a role in hair follicle proliferation. Follicle morphogenesis requires both activation of ectodermal proliferation and also remodeling of intercellular connections. One possibility is that β-catenin's role in these two processes is inversely related, such that when β-catenin is recruited as a transcription co-factor, it is weakened in its ability to perform at intercellular junctions. Further experiments will be necessary to test this hypothesis. (See color plates.)

summarizing these key points is outlined in Figure 6. While further studies will be needed to further test this model, a dual role for β-catenin stabilization in both activation of proliferation and inhibition of cell-cell adhesion poses a compelling argument for why the *Wnt* pathway is often implicated in developmental processes involving these two diverse functions. In support of this notion is the observation that an N-terminally truncated form of β-catenin suppresses intercellular adhesion and tubule formation in cultured MDCK kidney epithelial cells (Barth et al., 1997; Pollack et al., 1997).

VII. Summary

A number of fascinating questions remain unaddressed in the realm of skin biology. We still know very little about the mechanisms that set up the patterning of hair follicles over the surface ectoderm, or about the precise signalling pathways involved in mesenchymal-epithelial interactions during hair development and differentiation. Studies over the past 10 years have implicated both the *notch* and *sonic hedgehog* pathways in these processes (Chen et al., 1997; Chiang et al., 1999; Crowe et al., 1998; Kopan and Weintraub, 1993; Nohno et al., 1995; Oro and Scott, 1998; Powell et al., 1998; St. Jacques et al., 1998). Furthermore, we know that members of the fibroblast growth factor and bone morphogenic protein families are also involved in mesenchymal-epithelial cues required for follicle morphogenesis, hair cycling, and/or follicle differentiation (Hebert et al., 1994; Jung et al., 1998; Kratochwil et al., 1996; Noramly and Morgan, 1998; Rosenquist and Martin, 1996; Song et al., 1996). However, it is not clear precisely how these pathways and factors are involved and how they might also interact with the *wnt* pathway in regulating hair follicle patterning and morphogenesis. These areas are currently centers of activity in the field, and answers will undoubtedly emerge with the flurry of new experiments presently being conducted.

Another important issue is the residence of stem cells within the skin. While it is clear that the epidermis contains a population of cells with extraordinary proliferative capacity (Jones and Watt, 1993; Jones et al., 1995), their precise location in most body regions of the skin remains unclear. Similarly, while the bulge hypothesis has received considerable attention and support as the residence of the hair follicle stem cells (Cotsarelis et al., 1989; Lavker et al., 1993), the outer root sheath and the

matrix of the follicle have also been postulated as potential homes for these critical cells (Oliver and Jahoda, 1988; Rochat et al., 1994). An equally challenging issue for the future is the extent to which skin stem cells might retain pluripotency, able to choose between an epidermal or hair follicle cell fate. Our recent studies implicating a Wnt pathway provide a starting point for exploration.

I have discussed a number of recent insights that have surfaced concerning transcriptional regulation in the epidermis. Additionally, besides Lef1/β-catenin, there are a number of transcription factors that have been identified that are likely to regulate key aspects of hair follicle differentiation and gene expression. The most interesting of these include a member, Whn, of the winged-helix transcription factors, recently been shown to be the defect underlying the *nude* mouse phenotype (Nehls et al., 1994; Segre et al., 1995) and the zinc finger transcription factor responsible for the *hairless* phenotype in mice and in humans (Ahmad et al., 1998; Cachon-Gonzalez et al., 1994). A major area for future study will be to elucidate the upstream and/or downstream targets of Lef1/β-catenin, *hairless*, and *whn*.

Let me close by returning to the issues of structure and function in the skin and to the underlying genetic basis of skin disorders. It is surprising that despite nearly 20 years of molecular genetics and its application to skin biology, we still know very little about the molecules and pathways involved in the acquisition of the epidermal barrier, the very purpose of the epidermis. Biochemical studies have given us clues as to the most important lipids involved (for review, see Proksch et al., 1993), and recent studies suggest that barrier function may in part be regulated through action of the steroid hormone superfamily of receptors (Attar et al., 1997; Hardman et al., 1998). A major research effort is now needed to begin to decipher the transcriptional regulation and the complex pathways involved in lipid synthesis and packaging. Additional emphasis is needed to explore how the epidermis shuts down its metabolic activity once the enzymes and proteins necessary to create the lipid barrier have been made, and then finally, how the lipids are secreted and organized to create the Saran Wrap that covers our body surface, protecting us against harmful organisms from the outside and retaining our bodily fluids. As we face these new challenges and begin to unravel the mysteries still kept secret beneath the beauty of the skin, new insights will emerge not only into the biology of the skin but also into its genetic diseases.

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