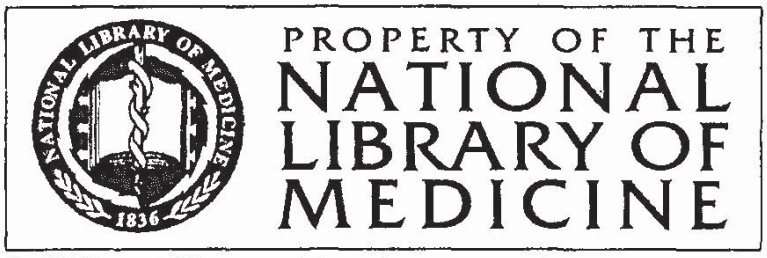

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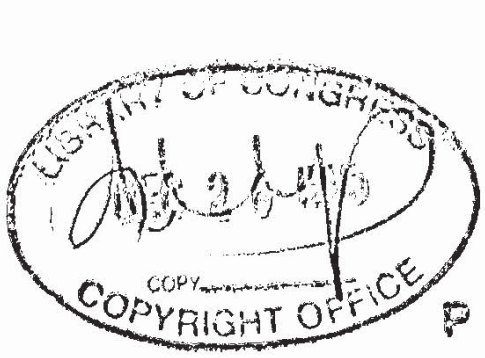

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KERATINS AND THE SKIN

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KEY WORDS: keratin filaments, genetic disease, epidermis, hair follicles, multigene family

CONTENTS

ABSTRACT

Keratins are the major structural proteins of the vertebrate epidermis and its appendages, constituting up to 85% of a fully differentiated keratinocyte. Together with actin microfilaments and microtubules, keratin filaments make up the cytoskeletons of vertebrate epithelial cells. Traced as far back in the evolutionary kingdom as mollusks, keratins belong to the superfamily of intermediate filament (IF) proteins that form α-helical coiled-coil dimers which associate laterally and end-to-end to form 10-nm diameter filaments. The evolutionary transition between organisms bearing an exoskeleton and those with an endoskeleton seemed to cause considerable change in keratin. Keratins expanded from a single gene to a multigene family. Of the ~60 IF genes in

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the human genome, half encode keratins, and at least 18 of these are expressed in skin. Vertebrate keratins are subdivided into two sequence types (I and II) that are typically coexpressed as specific pairs with complex expression patterns. The filament-forming capacity of a pair is dependent upon its intrinsic ability to self-assemble into coiled-coil heterodimers, a feature not required of the invertebrate keratins (Weber et al 1988). Approximately 20,000 heterodimers of type I and type II keratins assemble into an IF. Mutations that perturb keratin filament assembly in vitro can cause blistering human skin disorders in vivo. From studies of these diseases, an important function of keratins has been unraveled. These filaments impart mechanical strength to a keratinocyte, without which the cell becomes fragile and prone to rupturing upon physical stress. In this review, studies on the pattern of expression, structure, and function of skin keratins are summarized, and new insights into the functions of these proteins and their involvement in human disease are postulated.

SKIN AND ITS PROGRAMS OF EPITHELIAL DIFFERENTIATION

The single-layered embryonic ectoderm of mammals receives mesenchymal cues that specify its programs of differentiation. Early cues influence epidermal cell fate, and later signals influence the ectodermal cell to become epidermis vs hair follicle. These later cues happen shortly after stratification, where condensates of specialized mesenchyme, referred to as dermal papilla (anlage) cells, form in a dotted pattern beneath the embryonic basal layer (Figure 1). Where contact is made, basal cells of the epidermis differentiate downward to craft what will ultimately be the hair follicles of the mammalian skin. In the absence of these mesenchymal-epithelial interactions, basal cells commit to an epidermal cell fate.

At the single-layer stage, ectodermal proliferation occurs laterally, with the mitotic plane perpendicular to the embryo surface (Figure 2). Upon stratification, mitotic activity occurs in all layers, with the mitotic plane often parallel

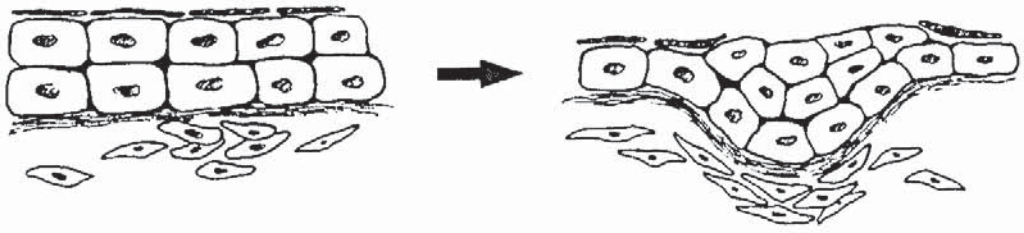

Figure 1 The choice between epidermis and hair follicles during embryonic development in the skin. Shortly after stratification, condensates of specialized mesenchyme, or dermal papilla anlage, assemble beneath the embryonic basal layer of the skin. These condensates provide as-yet-undetermined external cues that stimulate basal cells to migrate downward and form a primary hair germ. This morphogenic process eventually gives rise to an adult hair follicle.

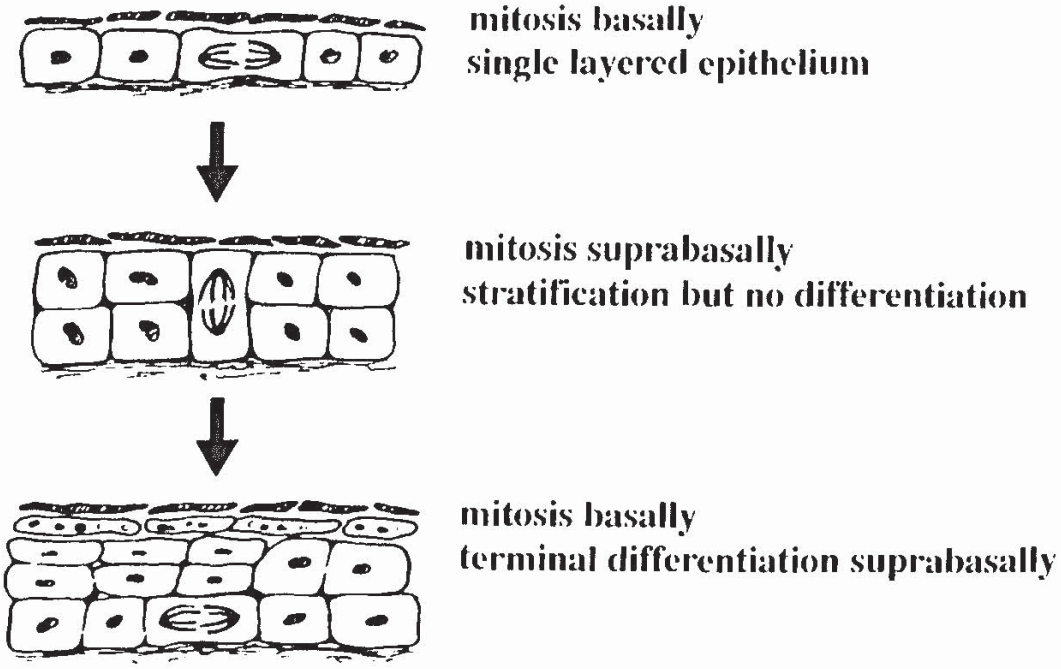

Figure 2 Cell division during embryonic development in the skin. At the single-layer epithelial stage, ectodermal proliferation occurs laterally, with the mitotic plane perpendicular to the embryo surface. The mitotic plane soon shifts 90°, causing stratification. Suprabasal mitoses are necessary during the time that the skin surface is rapidly expanding. Later, the mitotic plane reverts back, allowing dividing cells to move only laterally. At this stage, signs of differentiation appear in the suprabasal layers.

to the embryo surface (Figure 2). Suprabasal mitoses occur during the time that the skin surface undergoes rapid expansion. Later, as suprabasal cells display morphological signs of differentiation, mitotic activity becomes restricted to a single layer of basal cells, with the mitotic plane reverting to a perpendicular orientation. This pattern persists in postnatal epidermis.

In the adult, a basal epidermal cell responds to an as-yet-unidentified trigger of terminal differentiation. When it ceases to divide and begins its journey to the skin surface, it alters its adhesive properties, evoking changes that are likely to be central to the control of the differentiative program (for a recent review, see Watt et al 1993). In transit, the cell undergoes a series of morphological and biochemical changes culminating in the production of dead, flattened, enucleated squames, which are sloughed from the surface, continually replaced by inner cells differentiating outward (Figure 3). The trek takes 2 to 4 weeks in humans and continues throughout the life of the individual.

In the adult hair follicle, differentiation is far more complex than in the epidermis (Figure 4; Hardy 1992). The hair is surrounded by two sheaths, an outer root sheath (ORS), continuous with the epidermis but thought to have its own compartment of stem cells (Rochat et al 1994), and an inner root sheath

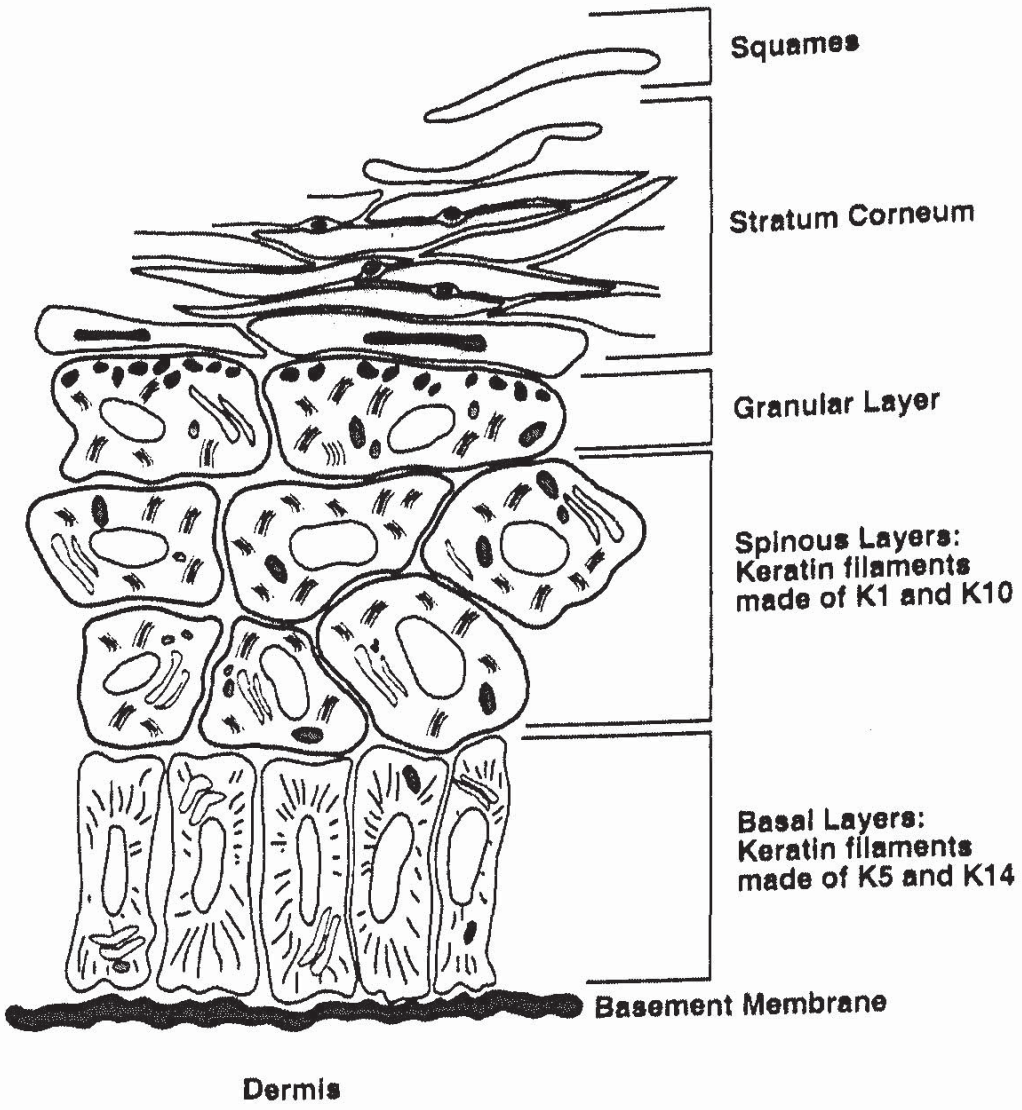

Figure 3 Terminal differentiation in adult epidermis. The diagram illustrates the four stages of terminal differentiation and the pattern of keratin expression. All cells above the innermost, i.e. basal, layer of the epidermis are considered to be suprabasal.

(IRS), whose cells are derived from the same precursors as the hair shaft. The ORS is composed of multiple layers, with the outermost layer being the least differentiated (Coulombe et al 1989). In the lower part of the ORS, cells move upward and inward as they differentiate, whereas in the upper part of the follicle, movement is largely inward, and differentiation more closely resembles that of the epidermis. The IRS is composed of three layers: the outer Henle's layer, the Huxley's layer, and the cuticle of the IRS. These three layers degenerate in the upper portion of the follicle. Another layer of cuticle forms the casing of the hair shaft and remains with it as it breaks through the skin surface (Figure 4).

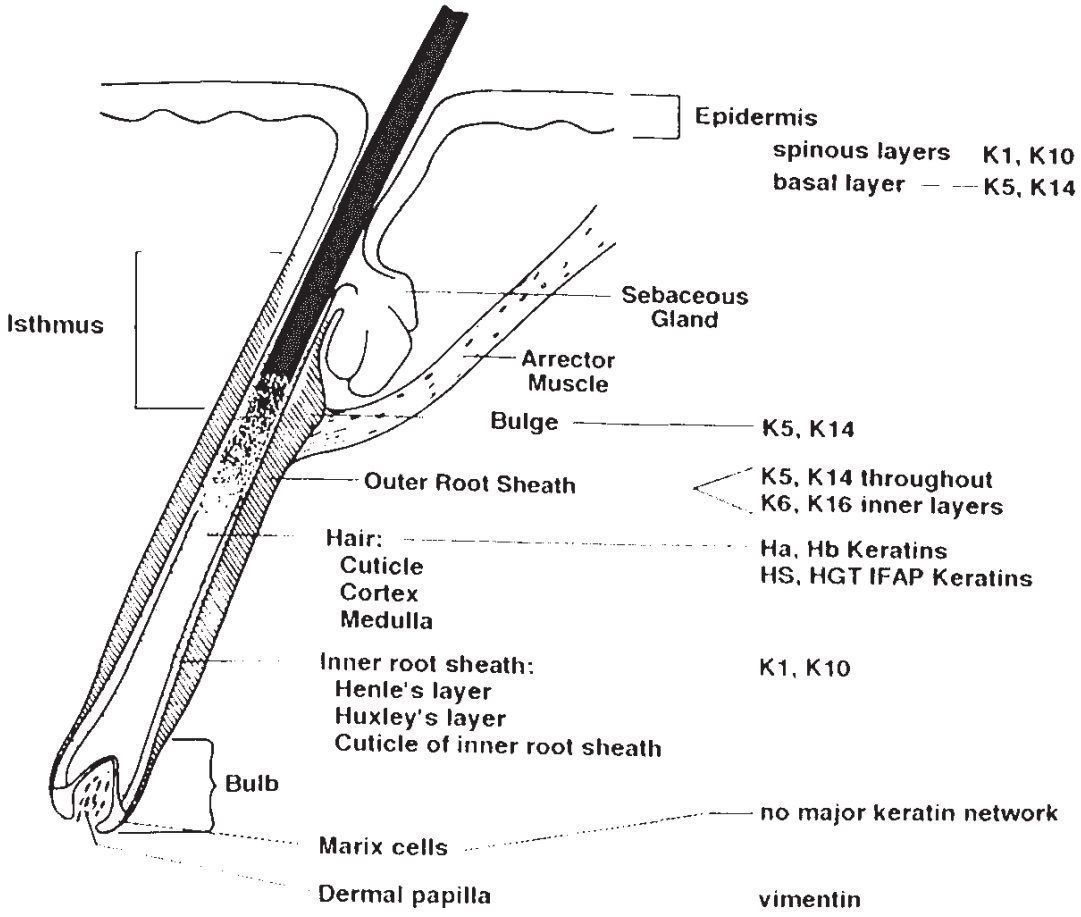

Figure 4 Terminal differentiation in the hair follicle. In the growth phase of the hair cycle, cells from the dermal papilla interact with proliferating matrix cells. Under an unidentified trigger, matrix cells cease to divide, begin to migrate upward, and commit to at least five concentric rings of differentiated states: The inner root sheath (IRS), consisting of Henle's layer, Huxley's layer, and outer cuticle, guides the hair shaft as it emerges from the immature cortex cells. The shaft consists of the inner cuticle and medulla. The outer root sheath (ORS) is contiguous with the basal epidermal layer and has a proliferating compartment distinct from matrix cells. These cells move upward and inward as they grow and differentiate. The bulge (see text) is a possible silo for stem cells. The keratins expressed in different cells of the hair follicle are indicated according to the nomenclature of Moll et al (1982).

The cells of the IRS and the hair shaft are thought to arise from upward modes of differentiation that are controlled by the adult dermal papilla cells, which maintain a condensate at the base of the follicle. The precursors of the hair shaft and IRS are matrix cells, which are relatively undifferentiated epithelial cells that surround the dermal papilla to form the hair bulb. In their mitotically active state, matrix cells maintain close association with the dermal papilla. As they move upward and away from this compartment, they cease to divide and begin to differentiate. These postmitotic cortical cells then give rise to the hair shaft as transcriptional activity comes to a halt.

Hairs follow rhythmic periods of growth and quiescence. In anagen, matrix cells are highly mitotically active, and the differentiating hair shaft moves upward at about 0.3–0.4 mm/day in humans (Kobori & Montagna 1976). Matrix cells are inactive in catagen, a period where the hair follicle degenerates and regresses. This is followed by telogen, the period of rest in the hair cycle. In humans, the resting period is variable: relatively short for scalp hairs, and longer for body hairs. The hair cycle is controlled in part by FGF5, a member of the fibroblast growth factor (FGF) family. Mice homozygous for a null FGF5 mutation have an extended cycle, resulting in the production of unusually long hairs (Hebert et al 1994).

Throughout an individual's life, the epidermis and hair follicles must maintain a balance of dividing and differentiating cells. Given the continuous renewal programs and ability to respond to injury, it is not surprising that these structures have reservoirs of cells capable of generating tremendous proliferation. The population of stem cells in the epidermis is likely to reside within the basal layer itself, and as judged by analysis of newborn human foreskin keratinocyte cultures, there is one clonal subtype, holoclones, whose cells possess extraordinary proliferative potential (>100 doublings) (Barrandon & Green 1987, Jones & Watt 1993, Jones et al 1995). Holoclones thus have the capacity to generate enough cells from a single clone to completely cover an adult human (Rochat et al 1994 and references therein). In the follicle, matrix cells are able to amplify as long as they maintain contact with dermal papilla condensates. These cells could be the source of stem cells for the IRS, cuticle, and hair shaft. However, follicle stem cells have also been hypothesized to reside in the bulge, a region at the midpoint of the follicle where the arrector pili muscle attaches (Cotsarelis et al 1990). This region contains 95% of the keratinocyte colony-forming cells isolated from rat vibrissae, and these cells are slow cycling, features generally ascribed to stem cells (reviewed in Rochat et al 1994). The location is attractive because the bulge effectively resides outside the hair follicle, which periodically degenerates. Cyclic stimulation of new stem cells in the bulge could then take place as the hair regresses upward, perhaps bringing dermal papilla cells with it (Cotsarelis et al 1990). The nature of the epithelial-mesenchymal interactions that control the complex differentiation programs in the follicle are not yet known, although keratinocyte growth factor (KGF), another member of the FGF family, has been implicated (Finch et al 1989, Guo et al 1993).

PATTERNS OF KERATIN EXPRESSION IN EMBRYONIC AND ADULT MAMMALIAN SKIN

The epidermis and its appendages devote the majority of their protein synthesizing machinery to making keratins. Figure 5 provides a schematic of a

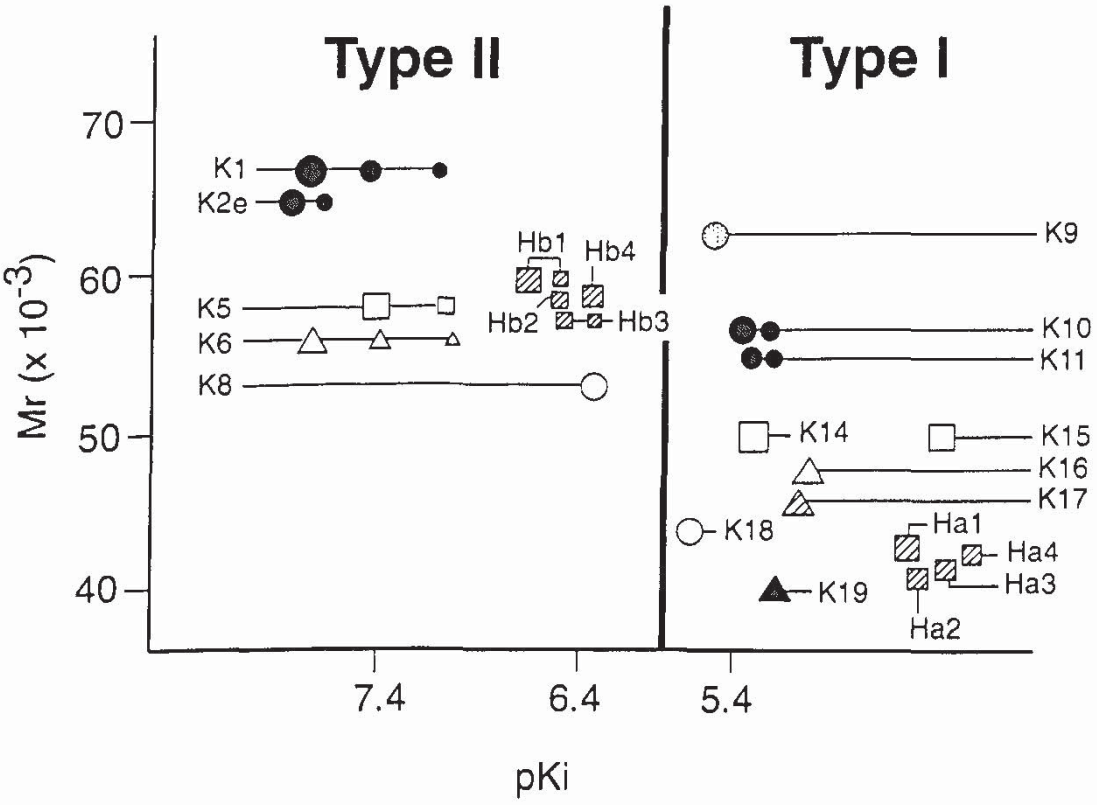

Figure 5 Two-dimensional gel profile of keratins expressed in the skin. The schematic illustrates the electrophoretic mobilities and isoelectric points of the various keratins found in the skin at different stages of development and differentiation. Keratins are expressed as specific pairs of type I and type II proteins, which form obligatory heteropolymers. Each pair is designated by like symbols, and the patterns of keratin expression are described in the text. The smaller sizes of like symbols indicate minor isoelectric variants of the same keratin (Expression patterns are according to Moll et al 1982.)

two-dimensional gel, showing the type, size, and approximate isoelectric points of human skin keratins, which are divided into types I and II based on isoelectric point and sequence. Figures 3 and 4 indicate the pattern of expression of the major keratins of adult mammalian epidermis and hair follicles. K8 and K18 are the first skin keratins expressed, coincident with the emergence of a single-layered ectoderm. These keratins are typically associated with adult simple epithelial tissues and are not characteristic of adult stratified squamous epithelia. K5 and K14 are then induced (E9.5 in mice) in a defined pattern that is influenced by mesenchyme (Byrne et al 1994). As judged by expression of a K5 promoter driven β-galactosidase transgene in mice, it is not until E14.5 that the entire surface ectoderm expresses these genes. In embryo and adult mice, K5 and K14 mRNAs seem to be restricted to cells that maintain their proliferative capacity (Byrne et al 1994 and references therein). A minor type I keratin, K15, is also expressed in basal keratinocytes (Lloyd et al 1995). As basal cells differentiate in adult skin, they downregulate expression of K5, Kl4,

and K15 and induce new sets of differentiation-specific keratins (Fuchs & Green 1980, Moll et al 1982, Sun et al 1984, Lloyd et al 1995). Most body regions express K1 and K10 suprabasally, along with a second type I keratin, K11. K2e is also quite broadly expressed suprabasally, but its production is delayed relative to K1 and K10 (Collin et al 1992). K9 is confined to suprabasal palmo and plantar skin (Fuchs & Green 1980, Langbein et al 1993). K6 and K16 are unusual in that they are induced suprabasally during wound healing, upon retinoic acid treatment, or in hyperproliferative diseases of the skin, including various skin cancers (Sun et al 1984). K6, K16, and K17 are also induced when skin keratinocytes are cultured in vitro. The functional significance of the multiplicity of keratins has not yet been resolved; however, the assembly properties of keratins differ as do their differential interactions with IF-associated proteins.

Keratin expression in the hair follicle is as complex as its differentiation programs suggest (Figure 4). In the ORS, as cells differentiate and move inward, they maintain mitotic activity, upregulate expression of K5 and K14, and also initiate expression of K6 and K16. In the IRS, K1 and K10 are expressed. The IF network in the matrix cells of the hair follicle has been difficult to discern either ultrastructurally or biochemically. K19 and possibly very low levels of K14 have been the only keratins identified in the matrix, and whether they produce a bona fide IF network remains to be determined. As these cells differentiate, however, they initiate abundant expression of Ha and Hb keratins, which are exclusive to the cortex of the follicle (Lynch et al 1986, Heid et al 1986, Moll et al 1988, Kopan & Fuchs 1989, Rogers & Powell 1993). In addition to Ha and Hb keratins, there are high sulfur (HS) and high glycine-tyrosine (HGT) proteins, which are also called keratins. These proteins share no sequence or structural homology to the IF keratins, but rather they are small IF-associated proteins that seal together the IFs into large macrofibrillar structures. Expression of the Ha/Hb, HS, and HGT keratins in the cortex is complex, and not all of the genes encoding these proteins are induced simultaneously (Rogers & Powell 1993).

REGULATION OF KERATIN GENE EXPRESSION IN THE SKIN

All keratins seem to be encoded by separate genes, and as yet, no evidence of differential splicing has been reported. Human K14 was the first keratin whose cDNA was cloned (Hanukoglu & Fuchs 1982) and whose gene was sequenced (Marchuk et al 1984). Following these studies, a flood of additional epidermal and hair follicle cDNA and gene sequences were reported (for review, see Fuchs & Weber 1994). As judged by nuclear run-off experiments, expression of skin keratin mRNAs is largely regulated at the transcriptional level (Stell-

mach et al 1991). Transgenic mouse studies have revealed that for many skin keratin genes, the sequences involved in regulating their expression reside in the 5′ upstream sequences of the genes (Vassar et al 1989, Bailleul et al 1990, Powell et al 1992, Takahashi et al 1994). A knowledge of the major transcription factors controlling skin keratin gene expression is of central importance in the quest to elucidate the molecular mechanisms underlying keratinocyte specificity and epidermal and follicle differentiation.

Among the candidates implicated in orchestrating epidermal gene expression is the sequence 5′-GCCTGCAGGC-3′, first identified 5′ from the TATA box of vertebrate K14 genes (Leask et al 1990, Snape et al 1990). For the K14 gene, the sequence acts in synergy with a distal element to regulate transcription in keratinocytes (Leask et al 1990). Epidermal nuclear extracts contain a protein(s) that binds to this sequence and cross-reacts with antibodies against the transcription factor AP2 (Leask et al 1990, Snape et al 1991). AP2-binding sites have now been found in the promoters of most epidermal- and some hair-specific genes, and where tested, they are functionally important for gene expression (Leask et al 1990, Snape et al 1990, Byrne & Fuchs 1993 and references within).

During embryogenesis, multiple AP2 mRNAs are synthesized in the skin (Snape et al 1991 and references within; Buettner et al 1993), and an AP2 cRNA recognizing these forms hybridizes to tissues of ectodermal and neural crest lineages (Mitchell et al 1991). As judged by in situ hybridizations of whole mouse embryos, the patterns of AP2 mRNA on the embryo surface are strikingly similar to and precede by about 1 day those patterns of K5 and K14 mRNAs (Byrne et al 1994). In contrast, a dominant negative inhibitor of AP2, referred to as AP2B (Buettner et al 1993), is not in the ectoderm of developing skin (Byrne et al 1994). Thus AP2 mRNAs are positioned temporally and spatially to play a role in controlling gene expression during skin development and differentiation.

How important is AP2 in controlling epidermal-specific gene expression? In at least one case, AP2 appears to be central. Thus recombinant AP2A (the active form of AP2) can impart to cultured hepatocytes the ability to express K5 and K14 promoter-driven transgenes when cotransfected into these cells (Byrne et al 1994). AP2B counteracts these effects. However, the mere presence of AP2 does not mandate the expression of endogenous K5 and K14 genes in cultured cells or in animals (Leask et al 1990), and thus AP2 cannot be the key to unleashing the cascade of keratinocyte-specific genes. Nevertheless, these findings do suggest that the AP2 forms present in embryonic ectoderm are likely to play a role in K5 and K14 activation in vivo, and in some cells, the absence of these factors may be sufficient to maintain these genes in an inactive state.

Members of the AP1 family of transcription factors are also found in the

epidermis of skin. These include junB (Wilkinson et al 1989) and cfos (Smeyne et al 1992 and references therein) in postnatal skin, and fosB in embryonic epidermis (Redemann-Fibi et al 1991). JunB and cfos reside primarily in the differentiating layers of epidermis, implicating these members of the AP1 family in regulating differentiation-specific functions in skin. Consistent with this notion is the finding that promoters active in terminally differentiating keratinocytes contain AP1 sites (Lu et al 1994 and references within; see also Casatorres et al 1994). Among the best evidence that these sites are functional comes from studies on the AP1 site 3′ to the K1 gene, which has been implicated in mediating the calcium-inducible, differentiation-specific expression of this gene (Lu et al 1994).

A number of other factors have been localized to the epidermis, although their roles in controlling keratinocyte-specific gene expression have not been fully elucidated. One of these proteins is a zinc finger protein, basonuclin, which is expressed in the basal layer of epidermis (Tseng & Green 1994). Basonuclin is interesting in that it persists in cells that have withdrawn from the cell cycle, but it is absent in terminally differentiating cells. Basonuclin has been postulated to be a key to the switch controlling the balance between growth and differentiation in keratinocytes.

Several POU-specific proteins have also been found in epidermis. A new class II POU sequence, called Skn-1a but related or identical to Oct-11, was detected in epidermis and hair follicles (Anderson et al 1993). Skn-1a can specifically upregulate expression of a human K10-luciferase reporter gene, suggesting a role in terminal differentiation (Anderson et al 1993). XLPOU1, a *Xenopus* class III POU protein, is also expressed in adult skin (Agarwal & Sato 1991). XLPOU1 shares ~90% sequence homology with human Oct-6, which has been cloned from epidermal keratinocytes (Faus et al 1994). Oct-6 is intriguing in that it can act in both positive and negative fashions, depending on the gene and the tissue. In basal keratinocytes, Oct-6 suppresses keratin gene expression, and it may possibly have a role in downregulation of these genes during terminal differentiation (Faus et al 1994).

Researchers are just beginning to decipher the sequences and transcription factors involved in controlling the expression of the hair-specific keratin genes. A number of potential regulatory motifs have been identified on the basis of sequence comparisons (reviewed in Rogers & Powell 1993). One of these sites, 5′-CTTTGAAGA-3′, referred to as the HK-1 motif, was detected in four published hair keratin promoters. When the HK-1 sequence motif was seen to overlap with a sequence identical to the motif of a known pair of lymphoid enhancer factors, LEF-1 and TCF-1, it became apparent that all thirteen published hair keratin promoters contained LEF-1/TCF-1 binding sites (Zhou et al 1995). Further studies showed that LEF-1 plays a functional role in hair-specific morphogenesis and keratin gene expression. LEF-1 is expressed early

in the development of embryonic skin, and it is produced in cells that first induce hair-specific gene expression (Zhou et al 1995). Interestingly, LEF-1 knockout mice have no whiskers and few hair follicles, further strengthening the notion that LEF-1 is involved in follicle morphogenesis (van Genderen et al 1994).

LEF-1 belongs to the high-mobility group (HMG) family of proteins that are not conventional transcription factors, but rather act by bending DNA and altering chromatin to create a structure conducive for conventional transcription factor binding (Giese et al 1992). It has been postulated that in hair follicles, LEF-1 is involved in creating a DNA structure conducive to initiation, but not necessarily maintenance, of gene expression, and that additional factors are likely to be necessary for hair-specific gene expression (Zhou et al 1995). One possible candidate is a zinc finger protein recently shown to be the product of the *hairless* gene in mice (Cachon-Gonzalez et al 1994). Like the LEF-1 knockout mice, *hairless* mice show a marked reduction and malformation of hair follicles, suggesting that they may be involved in the same pathway.

What governs transcriptional regulation early in development? Taking cues from other systems, researchers have begun to investigate skin-specific expression of the Hox class of transcription factors, known to specify positional information in segmentally derived structures, as well as in appendages such as limbs. Although epidermis is not a segmental structure, it overlies and is influenced by a dermis, which is, in part, segmentally derived. During embryogenesis, patterning and regionalization of the epidermis is likely to be under the influence of both dermal and epidermal *Hox* genes. An example of the former may be the segmental expression of the Mox1 homeoprotein in E8.5 dermamyotome in mouse embryos, in a pattern that parallels E9.5 expression of basal epidermal keratin genes in the overlying ectoderm (Byrne et al 1994 and references therein). At later developmental stages, mesodermal gradients of Hox proteins have been found in epidermal appendages of other vertebrates (reviewed in Chuong 1993), which suggests that positional information directing skin appendage patterning is determined by dermal *Hox* genes, perhaps in a fashion analogous to their mode of action in body appendages.

Hox genes are also expressed temporally in the ectodermal component of skin. Early in development, *Hox* 2 gene family members are expressed in the branchial arches of the head (Hunt et al 1991), and later the pattern of transient expression of additional members of this family suggests a role in specifying the differentiation status of epidermis (Mathews et al 1993). This notion is strengthened by the recent discovery of two additional differentiation-activated homeoproteins, Xdll-3/Dlx-3 (Morasso et al 1993) and HOXC4 (Rieger et al 1994).

It is well established that retinoids influence patterns of homeobox gene

expression, and thus it is not surprising that programs of epithelial differentiation in the skin are intricately controlled by retinoids. Retinoic acid at high (10^{-6} M) concentration has long been known to have inhibitory effects on epidermal differentiation and on the expression of keratin mRNAs (Fuchs & Green 1981). Recently, lower concentrations of retinoic acid have been shown to have a beneficial effect on the differentiation process. The effects of retinoids on keratin expression are transcriptionally regulated (Stellmach et al 1991, Tomic-Canic et al 1992, Lu et al 1994), and keratinocytes express a number of retinoid receptors. These include retinoic acid receptors α and γ (RARα and RARγ) and RXRα (Viallet & Dhouailly 1994 and references therein). RARγ and RXRα are expressed in neonatal skin and in only a few other organs, whereas RARα is more broadly expressed. Developmentally, RARγ mRNAs appear in epidermis prior to RXRα mRNAs, and in neonatal skin, RARγ mRNAs are most abundant in the keratinizing layers of epidermis.

The control of gene transcription by RARs and RXRs is complex, involving a multitude of both indirect and direct mechanisms (see Kastner et al 1993). RARs can heterodimerize with thyroid hormone receptors and with RXRs, and at least some of these interactions change the DNA affinity and activity of RARs. RARs and/or RXRs can bind to thyroid response elements, retinoic acid response elements (RAREs), and retinoid X response elements (RXREs), and the repertoire of complex interactions is further expanded by the capacity of RARs and RXRs to interact with AP1 proteins. The direct binding of RARs to epidermal keratin genes has not yet been demonstrated, although RA-mediated biochemical changes in differentiation do appear to involve DNA sequences in keratin genes that are responsive to retinoids (Tomic-Canic et al 1992, Lu et al 1994).

The effects of retinoids on epidermal growth and differentiation have been best studied through the use of RAR mutants targeted to epidermal cells in culture (Aneskievich & Fuchs 1992, 1995) or epidermal cells in transgenic mice (Saitou et al 1995, Imakado et al 1995). Although the precise nature of the RAR mutations differ in these studies, all mutants behave in a dominant-negative fashion, causing selective inhibition of RAREs. In cultured keratinocytes, an RARα or RARγ amino-terminal truncation mutant produced a complete block of terminal differentiation, resulting in stratified layers of basal-like cells (Aneskievich & Fuchs 1992, 1995). In mice, K14-mediated expression of an RARα harboring a single point mutation led to a similar, albeit incomplete, block in terminal differentiation (Saitou et al 1995). When a suprabasal promoter (K1) was used to drive expression of an RARα carboxy-terminal truncation mutant in mice, only very subtle morphological changes occurred in skin, but the terminally differentiated cells were no longer able to perform their normal protective function (Imakado et al 1995). Collectively, these studies demonstrate that the effects of retinoids on epidermal

differentiation are mediated through RARs and that abnormalities in RARα and RARγ can lead to an inhibition of the epidermal differentiative pathway.

A number of other extracellular regulators are known to influence keratin gene expression. Factors that enhance epidermal growth such as TGFα, EGF, and members of the FGF family generally downregulate keratin expression, although in suprabasal cells, the genes encoding K6 and K16 are specifically upregulated by these factors (Jiang et al 1993). Factors that enhance differentiation, such as calcium, typically promote expression of the differentiation-specific keratin genes (Rothnagel et al 1993, Lu et al 1994). Considerable headway has been made in uncovering the factors and sequences that orchestrate tissue-specific gene expression in the epidermis and its appendages. In the coming years, a major focus will be to develop a more detailed understanding of how these many different transcription factors are integrated in a program of growth, differentiation, and development.

ASSEMBLY AND STRUCTURE OF KERATIN FILAMENTS

Why are keratins the major structural proteins at the body surface of vertebrates, including mammals? An answer comes from considering the structures of these proteins and their higher ordered associations. Keratin filaments of the epidermis and its appendages involve intra- and intermolecular associations that are among the most stable found in nature.

Keratin genes, like all cytoplasmic IF genes, originated from a primordial nuclear lamin (for review, see Fuchs & Weber 1994). Like all cytoplasmic IF proteins, keratins are richly α-helical polypeptides (Astbury & Street 1931, Pauling & Corey 1953, Crick 1953). They have a central 310-amino acid α-helical rod domain that is subdivided by three short nonhelical linker segments (Hanukoglu & Fuchs 1982, 1983; Geisler & Weber 1982). The rod segments are referred to as helices 1A, 1B, 2A, and 2B; the linker segments are referred to as L1, L1–2 and L2 (Conway & Parry 1990). The keratin rods are flanked by larger nonhelical domains referred to as the head (amino terminal) and tail (carboxy terminal) segments.

During the course of evolution, most likely in the transition from invertebrates to vertebrates, gene duplication and divergence resulted in two distinct types (I and II) of keratins (Weber et al 1988). Among keratins of a single type, the α-helical rods share 50–99% sequence identity, whereas keratins of opposite type display only ~30% homology in these regions (Hanukoglu & Fuchs 1982, 1983; Steinert et al 1983). The sequence identity is particularly high at the start of helix 1A and near the end of helix 2B, and these sequences are even highly conserved across the IF superfamily. In contrast, the head and

tail segments are highly divergent, often sharing no sequence homology even among keratins of the same type.

It has long been known that keratin filaments can assemble in vitro in the absence of auxiliary proteins or factors. Thus the information necessary to form a 10-nm keratin filament is intrinsic, contained within the primary sequence of the polypeptide. To begin to assemble a protein into a series of complex higher-ordered alignments, the structure of the monomeric building block must be extraordinarily stable. It is notable that the α-helical rod segments have numerous acidic and basic residues spaced four amino acids (aa) apart (Letai & Fuchs 1995). Such spacing is optimal for the formation of ionic salt bridges that can stabilize α-helices (Huyghues-Despointes et al 1993 and references therein), thereby providing a foundation for the first step in the assembly process, which for vertebrate keratins is the formation of coiled-coil heterodimers of type I and type II keratins (Coulombe & Fuchs 1990, Hatzfeld & Weber 1990, Steinert 1990).

To form a heterodimer, the ~50-nm long rod segments align in parallel and in register (Pauling & Corey 1953, Crick 1953, Hanukoglu & Fuchs 1983, Woods & Inglis 1984, Parry et al 1985, Quinlan et al 1986). In vitro, virtually any type I and type II keratin will dimerize and proceed to form 10-nm long filaments (Franke et al 1983, Hatzfeld et al 1987), but keratins do not heterodimerize with other IF proteins (McCormick et al 1991). The dimerization process is driven by the ability of the rods of the two polypeptide chains to intertwine in a coiled-coil fashion, a feature that was originally predicted from X-ray diffraction data (Astbury & Street 1931, Pauling & Corey 1953, Crick 1953) and later substantiated by sequence data and model building (McLachlan 1978, Conway & Parry 1990). The α-helical sequences of the rods contain repeats of hydrophobic amino acids, such that the first and fourth of every seven residues are frequently apolar. This provides a hydrophobic seal on the helical surface, enabling the coiling between two IF polypeptides. For the epidermal keratins, these interactions are remarkably strong. Heterodimers are formed even in the presence of 9 M urea and a reducing agent, an additional reflection of the stability of these IF building blocks (Coulombe & Fuchs 1990).

An additional nonrandom periodic distribution of acidic and basic residues in the rod has been suggested to play a role in intermolecular ionic associations. A series of zones of alternating acidic and basic residues seem to repeat at ~9.5-residue intervals along the molecule, or three times every 28 residues (Parry et al 1977, McLachlan & Stewart 1982). This charge periodicity may reflect the importance of electrostatic interactions in stabilizing associations between coiled-coil dimers or higher ordered structures (Parry et al 1977, Fraser et al 1985, Conway & Parry 1990). While the potential for intramolecular ion pairing within the α-helical segments is substantially greater than that for intermolecular pairing across helical segments, it is possible that IF assem-

bly may be in part facilitated through the switching of acidic and basic interactions from helix-stabilizing intrachain salt bridges to interchain ionic associations (Letai & Fuchs 1995).

Keratin dimers associate readily in an antiparallel fashion to form stable tetramers (Woods & Inglis 1984, Quinlan et al 1984). Whether dimer units in keratin or other IF tetramers are in register or are staggered has been controversial, and both unstaggered and staggered forms have been described (reviewed in Fuchs & Weber 1994). For the basal epidermal keratins, staggered and unstaggered tetramers form in the presence of 6 M urea and a reducing agent (Coulombe & Fuchs 1990). Different modes of dimer association have also been identified by chemical cross–linking data (Geisler et al 1992, Steinert & Parry 1993, Steinert et al 1993). Three antiparallel arrangements of dimers have been identified: (*a*) near half-staggered dimers placing coil 1B segments into approximate alignment (Woods & Inglis 1984, Stewart et al 1989, Steinert & Parry 1993, Steinert et al 1993); (*b*) near half-staggered dimers placing coil 2B segments into approximate alignment (Geisler et al 1992, Steinert & Parry 1993, Steinert et al 1993); (*c*) dimers in approximate register, without stagger (Steinert & Parry 1993, Steinert et al 1993).

Many of the insights into the higher-ordered molecular associations in IFs have been gained by molecular mutagenesis of IF subunits. A number of the early studies in this area were conducted with epidermal keratin mutations. Deletion mutagenesis studies indicate that the ends of the rod play a special role in IF assembly (Albers & Fuchs 1987, 1989; Lu & Lane 1990, Coulombe et al 1990, Wong & Cleveland 1990, Gill et al 1990, Raats et al 1990, 1991). Even subtle point mutations in these highly conserved sequences can have deleterious effects (Loewinger & McKeon 1988, Hatzfeld & Weber 1991, Letai et al 1992) that are more severe than proline mutations appearing more centrally in the rod (Letai et al 1992). It has been postulated that in the linear, head-to-tail arrays of dimers, the rod ends of one dimer may overlap slightly with the rod ends of an adjacent dimer, thereby accounting for their special importance in IF structure (Steinert & Parry 1993, Steinert et al 1993). A model that is consistent with chemical cross–linking, mutagenesis and electron microscopy studies aligns two adjacent strings of head-to-tail linked dimers in a half-staggered arrangement (Figure 6). Two of these so-called protofilaments then intertwine to form a protofibril (Aebi et al 1983), and approximately four protofibrils then intertwine to form a single 10-nm filament (Aebi et al 1983). Thus a cross-section of the 10-nm filament passes through 16 type 1 and 16 type II keratin molecules.

How do the nonhelical head and tail segments of the IF polypeptides fit into the model of 10-nm filament assembly? Despite extensive mutagenesis studies, the function and structure of these end domains remain unresolved. Even within the family of keratins, these domains vary greatly in length (~10–200 residues)

and amino acid composition, making it doubtful that the heads and tails of keratin IFs produce a common structure. Indeed, deletion analyses of tail segments, coupled with the finding that K19 is a nearly tailless keratin (Bader et al 1986), suggest that much of this portion of IF proteins is dispensable to IF structure. Also, some studies suggest that tails may play a role in governing lateral associations at the protofilament and protofibril level (reviewed in Fuchs & Weber 1994). In contrast, IF head domains have more prominent and diverse roles in filament structure, and roles in both lateral and end-to-end linkages have been suggested. For the keratins, the type II head domain seems to be particularly important to IF structure.

ASSOCIATIONS BETWEEN KERATIN IFS AND OTHER PROTEINS AND STRUCTURES: FORMING A CYTOPLASMIC IF NETWORK IN EPIDERMIS AND HAIR

The vertebrate epidermal cell does not simply consist of a bag of 10-nm filaments, but rather it has an extensive cytoskeletal network of filaments, spanning from the nuclear envelope to the plasma membrane (Georgatos et al 1987, Djabali et al 1991 and references therein). Recent studies have begun to shed light on the nature of the epidermal keratin association with two kinds of specialized plaques in the plasma membrane, which serve as attachment sites for keratin filaments. Desmosomes are specialized vertebrate cell-cell adherens junctions composed of desmogleins and desmocollins, which are members of the cadherin superfamily (Green & Jones 1990; for review, see Garrod 1993). Hemidesmosomes are specialized vertebrate cell-basement membrane adherens junctions composed of the $\alpha 6,\beta 4$ integrin and several other associated proteins (Green & Jones 1990, Garrod 1993). Keratin IFs appear to loop through the matrix of desmosomal and hemidesmosomal plaques rather than terminate within them.

Although the precise interactions between keratin filaments and desmosomes or hemidesmosomes remain unknown, a growing family of high molecular mass, membrane-associated proteins appears to be involved (for review, see Foisner & Wiche 1991, Green et al 1992). The first of these members to be discovered, desmoplakin I or DPI, has a long (~150 nm) coiled-coil rod domain, flanked by head and tail segments (Green et al 1992). The other members of this family, bullous pemphigoid antigen (BPAG1) and plectin, have similar secondary structures (Stanley et al 1981, Foisner & Wiche 1991, Tanaka et al 1991). These proteins do not have a transmembrane domain, but rather localize to the cytoplasmic surface of the desmosome (DPI, plectin) or hemidesmosome (BPAG1, plectin). Plectin is unusual in that it decorates IFs in addition to its membrane associations and is ubiquitously expressed.

Studies on DPI indicate that it is co-recruited with keratin filaments to the

sites of artificial gap junctions produced by transfected keratinocytes expressing a hybrid desmocollin-connexin protein (Troyanovsky et al 1993). Deletion mutagenesis studies on DPI suggest that the union between it and IFs might be mediated through the DPI tail segment (Stappenbeck & Green 1992). Similar studies on plectin suggest that the six repeat motifs in plectin's tail may be required for IF association (Wiche et al 1993). Such repeat motifs are found in the tail domains of all members of this family.

The association between desmoplakin and epidermal keratin IFs appears to be a direct one, as judged by recent in vitro binding studies using purified, bacterially expressed desmoplakin and epidermal keratins (Kouklis et al 1994). In vivo, the head segment of the type II epidermal keratins is likely to be exposed, providing it with the potential to associate with desmoplakin.

The most direct evidence that this small family of proteins plays a role in connecting the cytoskeleton to adherens junctions comes from recent gene targeting studies (Guo et al 1995). When the BAPG1 gene is ablated in mice, the basal epidermal cytoskeleton is cleanly severed from hemidesmosomes, weakening the mechanical integrity at the base of the cell. Unexpectedly, the ablation leads to severe sensory neuron degeneration, and the generation of *dystonia muscularum* (*dt*) mouse. It appears that separate neuronal and epidermal forms are encoded by BPAG1 (Guo et al 1995). While additional studies will be required to explore the nature of these defects, studies on the epidermal form suggest that BPAG1 proteins might play a role in cell migration.

How do these interactions change during growth, differentiation, and cell migration, e.g. as in wound-healing? Although an exact answer is not yet at hand, desmosomes and possibly hemidesmosomes assemble in a calcium-dependent fashion, which suggests that intracellular fluxes in calcium might regulate these contacts. This could impart dynamic and flexible properties to the interactions between adjacent cells as well as between a cell and its basement membrane (for review, see Garrod 1993). Interestingly, the plectin-IF alliance appears to be governed by kinases, and PKA, PKC, and cdc2 kinase may all be involved (Foisner et al 1991; G Wiche, personal communication). Phosphorylation is likely to control desmosomal and hemidesmosomal connections with keratin IFs (Stappenbeck et al 1994), and phosphorylation-mediated events may offer additional versatility to IF cytoskeletons.

In addition to attaching keratin filaments to desmosomes and hemidesmosomes, the cytoarchitecture of a keratinocyte is likely to be influenced by interactions with other IF-associated proteins (IFAPs). They include (*a*) filaggrin, a calcium-regulated protein expressed late in epidermal terminal differentiation that can laterally cross-link IFs into bundles (Presland et al 1992, Markova et al 1993); (*b*) trichohyalin, a large (>200 kDa) α-helical, calcium-regulated IFAP produced and retained in the cells of the hardened inner root sheath and hair shaft (Fietz et al 1993); and (*c*) hair-specific IFAPs, which

embed IFs in a matrix of globular glycine- and tyrosine-rich proteins (Fratini et al 1993) and sulfur-rich proteins (MacKinnon et al 1990). The wrapping of keratin filaments in cloaks of these different IFAPs implies that the cytoarchitecture of a basal epidermal cell is quite different from that of a terminally differentiating epidermal cell or from one of the many different cells within a hair follicle. It is likely that the IFAPs provide a means to further specify the properties of the cytoskeletons within keratinocytes of the skin and in so doing may tailor the keratin networks to suit the distinctive needs of these cells at various stages of development and differentiation.

KERATIN MUTATIONS AND BLISTERING HUMAN SKIN DISORDERS

Given that keratins are the major structural proteins of the epidermis and its appendages, the question arises as to whether there might be genetic skin diseases that have as their basis defects in keratin, perhaps in a fashion analogous to that of diseases such as sickle cell anemia and the thallassemias, which have as their basis defects in globins, i.e. the major structural proteins of the blood. In the past five years, this hypothesis has been tested and shown to be correct.

Epidermolysis Bullosa Simplex

Affecting ~1:50,000 in the population, epidermolysis bullosa simplex (EBS) is a rare genetic skin disease. It is typified by mechanical stress-induced blistering, resulting from intraepidermal rupturing of basal keratinocytes, with no discernible aberrations in the suprabasal layers (for review, see Fine et al 1991). The disease is typically autosomal dominant, although recessiveness has been reported, and clinical manifestations are usually present at birth. There are three major subtypes of EBS. Weber-Cockayne (W-C) EBS is the mildest form, where blisters occur primarily on the hands and feet. No gross abnormalities have been detected in the W-C EBS basal keratin filament arrays, in contrast to the other two subtypes. Koebner (K) EBS is intermediate in severity, and Dowling-Meara (D-M) EBS is the severest form. The major ultrastructural feature distinguishing D-M from other EBS subtypes is the occurrence of clumps or aggregates of keratin within the basal cell cytoplasm (Anton-Lamprecht 1983).

Early on in the studies of EBS, it was recognized that clumping of keratin precedes cell degeneration, an indication that it is an early event in the blistering process (Anton-Lamprecht 1983). Similar perturbations were detected in cultured D-M EBS keratinocytes, which resembled wild-type keratinocytes that had been transfected with a mutant K14 gene (Albers & Fuchs 1987, Kitajima et al 1989). When engineered to express a mutant human K14 that

severely disrupts IF assembly, transgenic mice displayed nearly all the symptoms of D-M EBS (Vassar et al 1991). In contrast, transgenic mice expressing a mildly disrupting K14 mutant exhibited features typical of W-C EBS (Coulombe et al 1991b). From these findings, it was clear that structural defects in the K14, and presumably K5, genes could generate an EBS phenotype, and that the degree to which a specific K14 or K5 mutant perturbed filament assembly correlated with corresponding severity of the EBS phenotype.

Following these early studies were genetic studies of human patients with EBS. Indeed, patients with EBS contained point mutations or small deletions in their K14 or K5 genes (Coulombe et al 199la, Bonifas et al 1991, Lane et al 1992). The locations of these mutations are illustrated in Figure 6 and listed in Table 1. The genetic defects of EBS families also mapped to human chromosomes 17 or 12 (Bonifas et al 1991, Ryynanen et al 1991, Chan et al 1993), at locations corresponding to the loci for epidermal type I and type II keratin gene clusters, respectively (Rosenberg et al 1991). Most D-M EBS cases analyzed have a single amino acid substitution, 125R:C/H, in the conserved amino end of the K14 α-helical rod (Coulombe et al 1991a, Stephens et al 1993, J Cheng & E Fuchs, unpublished data). Affected members of another D-M EBS family have a 475E:G mutation in the conserved carboxy end of the K5 rod (Lane et al 1992). Only two other severe cases of autosomal dominant EBS have been described. One is a rare homozygous 173K:N mutation in helix 1A of the K5 rod that gives rise to severe blistering but no tonofilament clumping (K Stephens, personal communication). The other is a D-M mutation in the splice donor site of intron 1 of the K5 gene, and this gives rise to a putative 30-amino acid deletion within the H1 domain of the head and helix 1A (A Hovnanian, personal communication). The K-EBS mutations appear to be more central in the α-helical segments of the rod (Figure 6; for additional references, see Fuchs 1994). Interestingly, many of the identified mutations are prolines, which by random mutagenesis have been shown to perturb IF assembly to a lesser extent than subtle mutations at the rod ends (Letai et al 1992).

Thus far four W-C mutations have been identified, and these localize to nonhelical segments, either the head domain of K5 (Chan et al 1993) or in the carboxy half of the linker L1–2 segment (Rugg et al 1993, Chan et al 1994b). All these mutations correlate with affected individuals, and they have not been found in unaffected family members or the normal population.

Functional evidence suggests that the keratin point mutations identified in these EBS cases are in fact responsible for generating the EBS phenotype (Coulombe et al 1991a, Letai et al 1993). When expressed in transfected human epidermal cells, the D-M EBS point mutants cause perturbations in the IF networks similar to those detected in cultured D-M EBS keratinocytes. When combined with their wild-type partner in vitro, bacterially expressed D-M point

Table 1 Summary of mutations found in patients with EBS, EH, EN-EH, and PPK

Gene	Disease	Mutation	Domain	References not in Fuchs et al 1994
K14	D-M EBS	R125H (2)	1A	
K14	D-M EBS	R125C (7)	1A	
K14	K EBS	G107Stop	H1	Rugg et al 1994
K14	K EBS	Y204Stop	1B	Chan et al 1994a
K14	K EBS	L384P	2B	
K14	K EBS	M272R	L12	
K14	K EBS	A247D	1B	
K14	K EBS	ΔE375	2B	
K14	W-C EBS	V270M	L12	
K14	EBS-rec	E144A	1A	
K5	D-M EBS	E475G	2B	
K5	D-M EBS	Δ30 aa	H1/1A	
K5	K EBS	L463P	2B	
K5	EBS-hom	K173N	1A	
K5	W-C EBS	I161S (2)	H1	
K5	W-C EBS	M327T	L12	
K5	W-C EBS	N329K	L12	
K5	W-C EBS	R331C	L12	
K10	EH	R156H (5)	1A	
K10	EN	R156H	1A	Paller et al 1994
K10	EH	R156C (3)	1A	
K10	EH	R156L	1A	
K10	EN	R156C	1A	Paller et al 1994
K10	EH	R156P	1A	
K10	EH	R156S	1A	
K10	EH	M150R	1A	
K10	EN	M150T	1A	
K10	EH	N154H	1A	
K10	EH	L161S	1A	
K10	EH	Y160D	1A	
K10	EH	Y160N	1A	
K10	EH	L442Q	2B	
K10	EH-mild	K439E	2B	
K1	EH	S185P	1A	
K1	EH	N187P	1A	
K1	EH	N187S	1A	
K1	EH	S192P	1A	
K1	EH	Y481C	2B	
K1	EH	E489Q	2B	
K1	EH-mild	L160P	H1	
K1	EH-mild	V154G	H1	
K1	PPK-mild	K731	V1	
K2e	PPK-mild	E493K (3)	2B	McLean et al 1994, Kremer et al 1994
K2e	PPK-mild	E187P	1A	Kremer 1994

K9	EPPK	M156V (2)	1A	Hennies et al 1994
K9	EPPK	N160Y	1A	
K9	EPPK	N160K	1A	
K9	EPPK	R162Q	1A	
K9	EPPK	R162W	1A	
K9	EPPK	Q171P	1A	Hennies et al 1994

mutants cause shortening of filaments in a fashion similar to the altered filaments formed with keratins isolated from D-M EBS keratinocytes. The D-M mutations are particularly severe, presumably because they occur in domains that are involved in multiple contacts within IFs, and particularly in end-to-end associations (see Figure 6). In contrast, the W-C L1–2 linker mutations often cause filament unravelling, rather than filament shortening, which suggests that lateral associations are more severely affected by mutations in the nonhelical linker domains (Chan et al 1994b).

The discovery of the same 161I:S mutation in the H1 head domain of K5 of two unrelated W-C EBS families was a surprise because this residue is not a known hot-spot for mutagenesis. At present it is unclear why, when mutated, this residue or others in the H1 domain of the type II epidermal keratins give rise to epidermolytic skin disorders. Mutagenesis studies and computer analyses agree that the H1 segment plays a role in IF structure (for review, see Conway & Parry 1988). In other IF proteins, serines in this region are often targets for phosphorylation by cdc2 and other kinases, leading to destabilization or disassembly of IF networks (Heald & McKeon 1990; for review, see Fuchs & Weber 1994). In this regard, it is interesting that the 161I:S mutation creates a potential protein kinase C site (Chan et al 1993). Recently we identified a 161I:N mutation at this same residue in a W-C patient, which suggests that structure and not phosphorylation may be more key in perturbing the cytoarchitecture of the basal keratinocyte (Y Chan & E Fuchs, unpublished data).

Finally, two rare cases of recessive, severe EBS have recently been studied (Chan et al 1994a, Rugg et al 1994). Both patients are homozygous for a K14 premature stop codon mutation, resulting in a failure to produce stable K14 protein. In the absence of its partner, K5 is downregulated, and the classical bundles of K5- and K14-containing filaments in basal cells are missing. In both cases, the consanguineous parents are normal, each harboring one copy of the null K14 mutation. Interestingly, the severity of EBS appears to be roughly comparable, irrespective of whether the keratin network is disrupted by autosomal dominant mutants, or whether it is ablated by recessive, premature termination codon mutations. This has also recently been demonstrated in mice ablated for the K14 gene (Lloyd et al 1995).

Epidermolytic Hyperkeratosis

Epidermolytic hyperkeratosis (EH) is an autosomal dominant disease that shares certain striking mirror-image similarities to EBS. The histopathology of EH reveals a normal basal epidermal layer but cytolysis in the suprabasal layers (for review, see Anton-Lamprecht 1983). As in EBS, clumps of keratin filaments and perinuclear shells of keratin aggregates can be found in suprabasal cells. These parallels between EBS and EH were noted many years ago (Anton-Lamprecht & Schnyder 1974).

At the time it was discovered that transgenic mice expressing a truncated K14 bore a striking resemblance to human EBS, it was predicted that EH would be a disorder involving mutations in K1 and K10 (Vassar et al 1991), which are induced as epidermal cells move outward toward the skin surface (Fuchs & Green 1980). It was soon demonstrated that a truncated human K10 gene in mice generates the pathobiological and biochemical characteristics of EH (Fuchs et al 1992). In addition, genetic mapping of EH families revealed linkage to the keratin clusters on chromosomes 12 and 17 (Compton et al 1992). Point mutations in the K1 and K10 genes of patients with human EH then provided even stronger evidence that EH is a K1/K10 disorder (Cheng et al 1992, Chipev et al 1992, Rothnagel et al 1992, 1993; for review, see Fuchs et al 1994).

Remarkably, one residue mutated in K10, 156R:H/C, is the equivalent arginine residue that when mutated in K14 gives rise to EBS (Cheng et al 1992, Rothnagel et al 1992, Chipev et al 1994). Thus it appears that the same mutation in a highly conserved residue of two genes can give rise to two distinct genetic diseases by virtue of the differential expression of the genes. The high frequency with which this residue is mutated in EBS and EH is due to two factors: (*a*) It is a residue that is critical for IF assembly (Heald & McKeon 1990, Cheng et al 1992, Chipev et al 1992); and (*b*) it is a residue that is a hot-spot for C to T mutagenesis by CpG methylation and deamination (Cooper & Youssoufian 1988).

The first case analyzed of extremely mild EH, sometimes referred to as ichthyosis bullosa of Siemens, was found to have a mutation in helix 2B of the K10 gene, outside the 10 amino acids of the putative rod overlap (Syder et al 1994). Additional mild cases have mutations in the rod end domains of K2e (McLean et al 1994; Kremer et al 1994 and references therein). Presumably, severely disrupting mutations in K2e cause milder cases of EH because cytolysis can only take place in the upper spinous layers, where K2e is expressed (Collin et al 1992).

Epidermal Nevi of the EH Type

Many cutaneous disorders manifest clinically in a mosaic pattern, often as alternating stripes of affected and unaffected skin along the body surface.

Referred to as lines of Blaschko (Blaschko 1901), these stripes do not track along vascular, neural, or lymphatic structures of skin. Although never tested, this patterning has been attributed to clonal proliferation of two genetically distinct cell populations arising from a postzygotic mutation during embryogenesis. Epidermal nevi of the epidermolytic hyperkeratosis type is one such disease that tends to follow Blaschko's lines of clinical mosaicism. These patients sometimes have offspring with generalized EH. Recently, it was shown that not only do the EH offspring of three EN patients have mutations in their K10 genes, but in addition, the patients are genetically mosaic for the keratin mutation, with the mutation existing only in lesional skin (Paller et al 1994).

Given the parallels between EH and EBS, it is interesting that no mosaic disorder analogous to epidermal nevi is known for EBS. It could be that a genetic mosaicism in the basal keratin genes does not manifest itself clinically in a mosaic fashion. It seems likely that within a mosaic basal layer, healthy cells would divide and move laterally into sites vacated by mutant degenerating cells. The layer would thus be disproportionately populated with wild-type cells, generating clinically normal skin despite the genetically abnormality. An exception might occur when the majority of the epidermal cells carry the K5 or K14 mutation, but in this case, a diagnosis of EBS would be probable, and the minor mosaicism might go undetected. In contrast to basal cells, suprabasal cells terminally differentiate in upward columns of cells. Hence, when a somatic mutation exists in a suprabasally expressed gene, no compensation by lateral migration is possible, and clinical mosaicism is observed.

Palmoplantar Keratoderma and Pachyonychia Congenita

In the past year, epidermolytic palmoplantar keratoderma (EPPK) was shown to be a keratin disorder (Reis et al 1994, Torchard et al 1994, Hennies et al 1994). EPPK patients display palmoplantar skin blistering due to cytolysis in suprabasal layers. Thus far EPPK mutations have been found in K9, the 63-kDa keratin expressed specifically in plantar and palmar skin. Based on parallels between W-C EBS and EPPK, some patients might also be expected to have mild defects in their K1 or K10 genes.

Even more recently, pachyonychia congenita (PC) has been added to the growing list of keratin disorders (McLean et al 1995). This group of autosomal dominant disorders are characterized by thickening of the nails and, in some cases, hair defects and palmoplantar hyperkeratosis. McLean et al (1995) discovered a N92:D mutation in helix 1A of K17 in one family with the Jackson-Lawler subtype of the disease and an L130:P mutation in helix 1A of K16 in the Jadassohn-Lewandowsky form of PC.

KERATIN IFS FUNCTION TO IMPART MECHANICAL INTEGRITY TO CELLS: POSSIBLE INSIGHTS INTO ADDITIONAL GENETIC DISORDERS OF KERATIN

For many years, the function(s) of IFs have remained obscure. Based on the knowledge that keratin mutations lead to cell fragility, a role in imparting mechanical integrity to cells now seems likely. The recent reports of recessive severe EBS (Rugg et al 1994, Chan et al 1994a) and K14 knockout mice (Lloyd et al 1995) provide the best evidence that the function of keratin filaments in epidermal cells is to impart mechanical integrity to cells, without which the cells become fragile and prone to rupturing upon mild physical trauma.

Keratins of the epidermal appendages such as hair also appear to function in providing mechanical strength to cells. Thus transgenic mice overexpressing a wild-type hair keratin gene have fragile cortical cells that lead to brittleness and hair breakage (Powell & Rogers 1990). The identification of human diseases of hair-specific keratin genes may be difficult due to the large number of these genes coexpressed in the cortex. However, mutant *Rex* (*Re*) mice with curly whiskers and bent hair shafts, and *denuded* (Re^{den}) and *bareskin* (*Bsk*) mice, which undergo hair loss after completion of the first hair cycle, map within the type I keratin cluster (Nadeau et al 1989 and references therein).

CONCLUSION AND PERSPECTIVES

It is becoming increasingly clear that keratins are the mechanical integrators of space for the epidermis and its appendages. Whether all keratins and other members of the IF family perform this function is questionable. Even though ablation of epidermal keratin networks are detrimental to skin function, ablation of some IF genes in mice can result in no obvious phenotype (Baribault et al 1993, Colucci-Guyon et al 1994). In many ways this is not surprising, because the degree to which the loss or perturbation of an IF network causes cell fragility and tissue degeneration would be expected to depend upon the tissue architecture and the extent to which a tissue is subjected to mechanical stress. The extent to which defects in other human IF genes result in IF network abnormalities and cell degeneration, e.g. in such diseases as amyotrophic lateral sclerosis (ALS) and some forms of familial cardiomyopathies, remains to be explored, and recent work from several laboratories is encouraging (Xu et al 1993, Cote et al 1993).

Future studies of the skin and keratin will likely focus on IFAPs and adherens junctions to learn more about how keratin networks form and how they change with development and differentiation. As both classical genetic approaches and gene ablation studies are conducted, the genetic bases underlying additional structural disorders of the skin will be elucidated. Finally, as the intri-

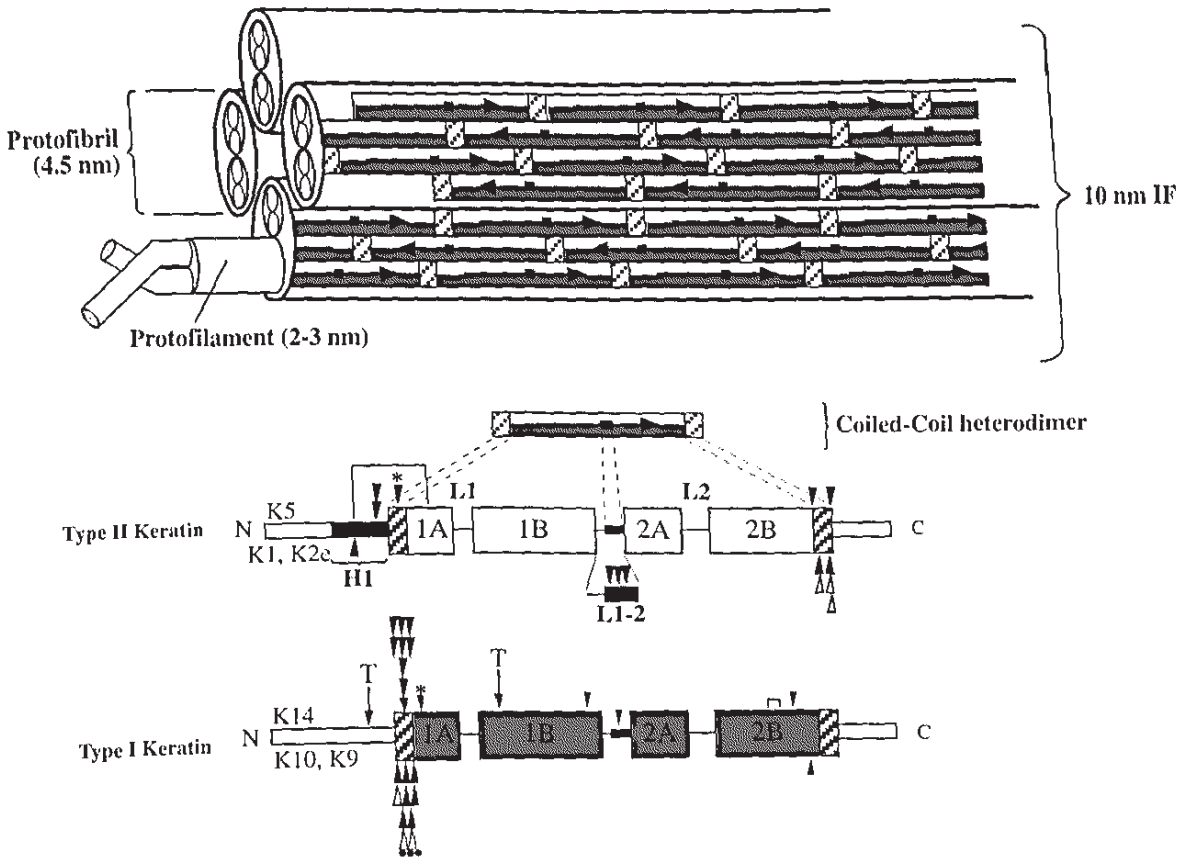

Figure 6 Model of keratin filament assembly and correlation between mutation location and disease severity in genetic disorders of keratin. (*Top*) Model is adapted from that previously described (Fuchs 1994; permission from *J. Cell Biol.*; see also Heins et al 1993, Steinert et al 1993, Fuchs & Weber 1994). Yellow boxes denote type II keratin rod segment of dimer; fuchsia boxes denote type I keratin rod segment of dimer; arrow indicates direction of polypeptides, from base (N-terminus) to tip (C-terminus); hatched boxes denote the highly conserved ends of helix 1A and 2B, which in the diagram are overlapping, as suggested by Steinert et al (1993). Note: In this model, unstaggered anti-parallel alignments of dimers arise at the level of protofibril-protofibril associations (Heins et al 1993). The small black bar in each arrow denotes the sequence in one coiled-coil dimer that is directly opposite the putative rod end-overlap in an adjacent dimer. (*Bottom*) Correlation between mutation location and disease severity in genetic disorders of keratin. Stick figures depict secondary structures of human type II and type I keratins (Hanukoglu & Fuchs 1982, 1983). Large boxes encompass the α-helical rod domains (1A, 1B, 2A, and 2B) interrupted by the short nonhelical linker segments (L1, L1-2, and L2). Hatched boxes denote conserved ends of the rod. Thinner bars denote nonhelical head and tail domains, with the H1 domain unique to type II keratins (Steinert et al 1993) shown as solid bar. Solid arrows over diagram denote positions of EBS mutations (D-M, red; K, green; W-C, blue; recessive/homozygous, asterisk). Solid arrowheads under diagram denote positions of EH K1/K10 mutations. Open arrowheads denote mutations in EH (K2e) or in EPPK (K9). Red bracket denotes 30 aa K5 deletion resulting from a spliced defect in a D-M EBS patient. Black bracket denotes single aa deletion in a K-EBS patient. Solid red balls denote mosaic K10 mutations in epidermal nevi patients of the epidermolytic hyperkeratosis type (Paller et al 1994). Two red Ts denote positions of premature termination codons in the two K14 severe recessive EBS cases (Rugg et al 1994, Chan et al 1994a).

cacies of epidermal-specific and hair-specific gene expression unfold, along with an understanding of how these processes are influenced by extracellular factors and mesenchymal signals, insights into other aspects of dermatology are likely to unravel.

Literature Cited

Aebi U, Fowler WE, Rew P, Sun TT. 1983. The fibrillar substructure of keratin filaments unraveled. *J. Cell Biol.* 97:1131–43

Agarwal VR, Sato M. 1991. *XLPOU 1* and *XLPOU 2*, two novel POU domain genes expressed in the dorsoanterior region of *Xenopus* embryos. *Dev. Biol.* 147:363–73

Albers K, Fuchs E. 1987. The expression of mutant epidermal keratin cDNAs transfected in simple epithelial and squamous cell carcinoma lines. *J. Cell Biol.* 105:791–806

Albers K, Fuchs E. 1989. Expression of mutant keratin cDNAs in epithelial cells reveals possible mechanisms for initiation and assembly of intermediate filaments. *J. Cell Biol.* 108: 1477–93

Aneskievich BJ, Fuchs E. 1992. Terminal differentiation in keratinocytes involves positive as well as negative regulation by RARs/RXRs at retinoid response elements. *Mol. Cell Biol.* 12:4862–71

Aneskievich BJ, Fuchs E. 1995. The A/B domain of truncated RARs can block differentiation and promote features of malignancy. *J. Cell Sci.* 108:195–205

Anderson B, Schonemann MD, Flynn SE, Pearse R, Singh H, Rosenfeld MG. 1993. Skn-1a and Skn-1i: two functionally distinct oct-2-related factors expressed in epidermis. *Science* 260:78–82

Anton-Lamprecht I. 1983. Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolysis, pathogenesis, heterogeneity, fetal manifestation, and prenatal diagnosis. *J. Invest. Dermatol.* 81(Suppl. 1):149s–56s

Anton-Lamprecht I, Schnyder UW. 1974. Ultrastructure of inborn errors of keratinization. *Arch. Derm. Forsch.* 250:207–27

Astbury WT, Street A. 1931. X-ray studies of the structures of hair, wool and related fibres. I. *Gen. Philos. Trans. R. Soc.* 23OA:75–78

Bader BL, Magin TM, Hatzfeld M, Franke WW. 1986. Amino acid sequence and gene organization of cytokeratin no. 19, an exceptional tail-less intermediate filament protein. *EMBO J.* 5:1865–75

Bailleul B, Surani MA, White S, Barton SC, Brown K, et al. 1990. Skin hyperkeratosis and papilloma formation in transgenic mice expressing a ras oncogene from a suprabasal keratin promoter. *Cell* 62:697–708

Baribault H, Price J, Miyai K, Oshima RG. 1993. Mid-gestational lethality in mice lacking keratin 8. *Genes Dev.* 7:1191–1201

Barrandon Y, Green H. 1987. Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-alpha and epidermal growth factor. *Cell* 50:1131–37

Blaschko A. 1901. Die Nervenverteilung in der Haut in ihrer Beziehung zu den Erkrankugen der Haut. In *Beilage zu den Verhandlungen der Deutschen Dermatologischen Gesellschaft,* VII, ed. Congress zu Breslau. Braumuller, Vienna, Austria

Bonifas JM, Rothman AL, Epstein EH. 1991. Epidermolysis bullosa simplex: evidence in two families for keratin gene abnormalities. *Science* 254:1202–5

Buettner R, Kannan P, Imhof A, Bauer R, Yim SO, et al. 1993. An alternatively spliced mRNA from the AP-2 gene encodes a negative regulator of transcriptional activation by AP-2. *Mol. Cell. Biol.* 13:4174–85

Byrne C, Fuchs E. 1993. Probing keratinocyte and differentiation specificity of the K5 promoter in vitro and in transgenic mice. *Mol. Cell. Biol.* 13:3176–90

Byrne C, Tainsky M, Fuchs E. 1994. Programming gene expression in developing epidermis. *Development* 120:2369–83

Cachon-Gonzalez MB, Fenner S, Coffin JM, Moran C, Stoye JP, Best S. 1994. Structure and expression of the *hairless* gene in mice. *Proc. Natl. Acad. Sci. USA* 91:7717–21

Casatorres J, Navarro JM, Blessing M, Jorcano JL. 1994. Analysis of the control of expres-

sion and tissue specificity of the keratin 5 gene, characteristic of basal keratinocytes. Fundamental role of an AP-1 element. *J. Biol. Chem.* 269:20489–96

Chan YM, Anton-Lamprecht I, Yu QC, Jackel A, Zabel B, et al. 1994a. A human keratin 14 "knockout": the absence of K14 leads to severe epidermolysis bullosa simplex and a function for an intermediate filament protein. *Genes Dev.* 8:2574–87

Chan YM, Yu QC, Christiano A, Uitto J, Kucherlapati RS, et al. 1994b. Mutations in the non-helical linker segment L1-2 of keratin 5 in patients with Weber-Cockayne epidermolysis bullosa simplex. *J. Cell Sci.* 107: 765–74

Chan YM, Yu QC, Fine JD, Fuchs E. 1993. The genetic basis of Weber-Cockayne epidermolysis bullosa simplex. *Proc. Natl. Acad. Sci. USA* 90:7414–18

Cheng J, Syder AJ, Yu QC, Letai A, Paller AS, Fuchs E. 1992. The genetic basis of epidermolytic hyperkeratosis: a disorder of differentiation-specific epidermal keratin genes. *Cell* 70:811–19

Chipev CC, Korge BP, Markova N, Bale SJ, DiGiovanna JJ, et al. 1992. A leucine to proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. *Cell* 70:821–28

Chipev CC, Yang JM, DiGiovanna JJ, Steinert PM, Markov L, et al. 1994. Preferential sites in keratin 10 that are mutated in epidermolytic hyperkeratosis. *Am. J. Hum. Genet.* 54:179–90

Chuong CM. 1993. The making of a feather: homeoproteins, retinoids and adhesion molecules. *BioEssays* 15:513–21

Collin C, Moll R, Kubicka S, Ooukayoun JP, Franke WW. 1992. Characterization of human cytokeratin 2, an epidermal cytoskeletal protein synthesized late during differentiation. *Exp. Cell Res.* 202:132–41

Colucci-Guyon E, Portier MM, Dunia I, Paulin D, Pournin S, Babinet C. 1994. Mice lacking vimentin develop and reproduce without an obvious phenotype. *Cell* 79:679–94

Compton JG, DiGiovanna JJ, Santucci SK, Kearns KS, Amos CI, et al. 1992. Linkage of epidermolytic hyperkeratosis to the type II keratin gene cluster on chromosome 12q. *Nat. Genet.* 1:301–5

Conway JF, Parry DAD. 1988. Intermediate filament structure: 3. analysis of sequence homologies. *Int. J. Biol. Macromol.* 10:79–98

Conway JF, Parry DAD. 1990. Structural features in the heptad substructure and longer range repeats of two-stranded alpha-fibrous proteins. *Int. J. Biol. Macromol.* 12:328–34

Cooper DN, Youssoufian H. 1988. The CpG dinucleotide and human genetic disease. *Hum. Genet.* 78:151–55

Cote F, Collard JF, Julien JP. 1993. Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell* 73:35–46

Cotsarelis G, Sun TT, Lavker RM. 1990. Label-retaining cells residue in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* 61:1329–37

Coulombe PA, Chan YM, Albers K, Fuchs E. 1990. Deletions in epidermal keratins leading to alterations in filament organization in vivo and in intermediate filament assembly in vitro. *J. Cell Biol.* 111:3049–64

Coulombe PA, Fuchs E. 1990. Elucidating the early stages of keratin filament assembly. *J. Cell Biol.* 111:153–69

Coulombe PA, Hutton ME, Letai A, Hebert A, Paller AS, Fuchs E. 1991a. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. *Cell* 66:1301–11

Coulombe PA, Hutton ME, Vassar R, Fuchs E. 1991b. A function for keratins and a common thread among different types of epidermolysis bullosa simplex diseases. *J. Cell Biol.* 115:1661–74

Coulombe PA, Kopan R, Fuchs E. 1989. Expression of keratin K14 in the epidermis and hair follicle: insights into complex programs of differentiation. *J. Cell Biol.* 109:2295–312

Crick FHC. 1953. The packing of alpha-helices: simple coiled-coils. *Acta Crystallogr.* 6: 689–97

Djabali K, Portier MM, Gros F, Blobel G, Georgatos S. 1991. Network antibodies identify nuclear lamin B as a physiological attachment site for peripherin intermediate filaments. *Cell* 64:109–21

Faus I, Hsu HJ, Fuchs E. 1994. Oct 6: a regulator of keratinocyte gene expression in stratified squamous epithelia. *Mol. Cell. Biol.* 14:3263–75

Fietz MJ, McLaughlan CJ, Campbell MT, Rogers GE. 1993. Analysis of the sheep trichohyalin gene: potential structural and calcium-binding roles of trichohyalin in the hair follicle. *J. Cell Biol.* 121:855–65

Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA. 1989. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science* 245:752–55

Fine JD, Bauer EA, Briggaman RA, Carter DM, Eady RAJ, et al. 1991. Revised clinical and laboratory criteria for subtypes of inherited epidermolysis bullosa. *J. Am. Acad. Dermatol.* 24:119–35

Foisner R, Traub P, Wiche G. 1991. Protein kinase A- and protein kinase C-regulated interaction of plectin with lamin B and vimentin. *Proc. Natl. Acad. Sci. USA* 88: 3812–16

Foisner R, Wiche G. 1991. Intermediate filament-associated proteins. *Curr. Opin. Cell Biol.* 3:75–81

Franke WW, Schiller DL, Hatzfeld M, Winter S. 1983. Protein complexes of intermediate-sized filaments-melting of cytokeratin compexes in urea reveals different polypeptide separation characteristics. *Proc. Natl. Acad. Sci. USA* 80:7113–17

Fraser RDB, MacRae TP, Suzuki E, Parry DAD, Trajstmann AC, Lucas I. 1985. Intermediate filament structures. 2. Molecular interactions in the filament. *Int. J. Biol. Macromol.* 7:258–74

Fratini A, Powell BC, Rogers GE. 1993. Sequence, expression, and evolutionary conservation of a gene encoding a glycine/tyrosine-rich keratin-associated protein of hair. *J. Biol. Chem.* 268:4511–18

Fuchs E. 1994. Intermediate filaments and disease: mutations that cripple cell strength. *J. Cell Biol.* 125:511–16

Fuchs E, Coulombe P, Cheng J, Chan YM, Hutton E, et al. 1994. Genetic bases of epidermolysis bullosa simplex and epidermolytic hyperkeratosis. *J. Invest. Dermatol.* 103: S25–S30

Fuchs E, Esteves RA, Coulombe PA. 1992. Transgenic mice expressing a mutant keratin 10 gene reveal the likely genetic basis for epidermolytic hyperkeratosis. *Proc. Natl. Acad. Sci. USA* 89:6906–10

Fuchs E, Green H. 1980. Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell* 19:1033–42

Fuchs F, Green H. 1981. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell* 25:617–25

Fuchs E, Weber K. 1994. Intermediate filaments: structure, dynamics, function, and disease. *Annu. Rev. Biochem.* 63:345–82

Garrod DR. 1993. Desmosomes and hemidesmosomes. *Curr. Opin. Cell Biol.* 5:30–40

Geisler N, Schunemann J, Weber K. 1992. Chemical cross-linking indicates a staggered and antiparallel protofilament of desmin intermediate filaments and characterizes one higher-level complex between protofilaments. *Eur. J. Biochem.* 206:841–52

Geisler N, Weber K. 1982. The amino acid sequence of chicken muscle desmin provides a common structural model for intermediate filament proteins. *EMBO J.* 1:1649–56

Georgatos SD, Weber K, Geisler N, Blobel G. 1987. Binding of two desmin derivatives to the plasma membrane and the site-specificity in intermediate filament-membrane interactions. *Proc. Natl. Acad. Sci. USA* 84:6780–84

Giese K, Cox J, Grooschedl R. 1992. The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Cell* 69:185–95

Gill SR, Wong PC, Monteiro MJ, Cleveland DW. 1990. Assembly properties of dominant and recessive mutations in the small mouse neurofilament (NF-L) subunit. *J. Cell Biol.* 111:2005–19

Green KJ, Jones JCR. 1990. *Cellular and Molecular Biology of Intermediate Filaments: Interaction of Intermediate Filaments With the Cell Surface*, pp. 147–74. New York: Plenum

Green KJ, Virata MLA, Elgart GW, Stanley JR, Parry DAD. 1992. Comparative structural analysis of desmoplakin, bullous pemphigoid antigen and plectin: members of new gene family involved in organization of intermediate filaments. *Int. J. Biol. Macromol.* 14:145–53

Guo L, Degenstein L, Dowling D, Yu Q-C, Wollman R, et al. 1995. Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and severe neurologic degeneration—the dystonia muscularum mouse. *Cell* 81: 233–44

Guo L, Yu QC, Fuchs E. 1993. Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. *EMBO J.* 12:973–86

Hanukoglu I, Fuchs E. 1982. The cDNA sequence of a human epidermal keratin: divergence of sequence but conservation of structure among intermediate filament proteins. *Cell* 31:243–52

Hanukoglu I, Fuchs E. 1983. The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. *Cell* 33:915–24

Hardy MH. 1992. The secret life of the hair follicle. *Trends Genet.* 8:159–66

Hatzfeld M, Maier G, Franke WW. 1987. Cytokeratin domains involved in heterotypic complex formation determined by in vitro binding assays. *J. Mol. Biol.* 197:237–55

Hatzfeld M, Weber K. 1990. The coiled coil of in vitro assembled keratin filaments is a heterodimer of type I and II keratins. Use of site-specific mutagenesis and recombinant protein expression. *J. Cell Biol.* 110:1199–210

Hatzfeld M, Weber K. 1991. Modulation of keratin intermediate filament assembly by single amino acid exchanges in the consensus sequence at the C-terminal end of the rod domain. *J. Cell Sci.* 99:351–62

Heald R, McKeon F. 1990. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell* 61: 579–89

Hebert JM, Rosenquist T, Gotz J, Martin GR. 1994. FGF5 as a regulator of the hair growth cycle: evidence from targeted and spontaneous mutations. *Cell* 78:1017–25

Heid HW, Werner E, Franke WW. 1986. The complement of native alpha-keratin polypeptides of hair-forming cells. A subset of eight polypeptides that differ from epithelial cytokeratins. *Differentiation* 32:101–19

Heins S, Wong PC, Muller S, Goldie K, Cleveland DW, Aebi U. 1993. The rod domain of NF-L determines neurofilament architecture, whereas the end domains specify filament assembly and network formation. *J. Cell Biol.* 123:1517–33

Hennies HC, Zehender D, Kunze J, Kuster W, Reis A. 1994. Keratin 9 gene mutational heterogeneity in patients with epidermolytic palmoplantar keratoderma. *Hum. Genet.* 93: 649–54

Hunt P, Wilkinson D, Krumlauf R. 1991. Patterning the vertebrate head: murine *hox 2* genes mark distinct subpopulations of premigratory and migrating neural crest. *Development* 112:43–50

Huyghues-Despointes BM, Scholtz JM, Baldwin RL. 1993. Helical peptides with three pairs of Asp-Arg and Glu-Arg residues in different orientations and spacings. *Protein Sci.* 2:80–85

Imakado I, Sunihisa I, Bickenbach JR, Bundman DS, Rothnagel JA, et al. 1995. Targeting expression of a dominant-negative retinoic acid receptor mutant in the epidermis of transgenic mice results in loss of barrier function. *Genes Dev.* 9:317–29

Jiang CK, Magnaldo T, Ohtsuki M, Freedberg IM, Bernerd F, Blumenbert M. 1993. Epidermal growth factor and transforming growth factor alpha specifically induce the activation and hyperproliferation-associated keratins 6 and 16. *Proc. Natl. Acad. Sci. USA* 90:6786–90

Jones PH, Harper S, Watt FM. 1995. Stem cell patterning and fate in human epidermis. *Cell* 80:83–94

Jones PH, Watt FM. 1993. Separation of human epidermal stem cells from transit-amplifying cells on the basis of differences in integrin function and expression. *Cell* 73:713–24

Kastner P, Leid M, Chambon P. 1993. Role of nuclear retinoic receptors in the regulation of gene expression. In *Vitamin A in Health and Disease*, ed. R Blonhoff, pp. 189–238. New York: Dekker

Kitajima Y, Inoue S, Yaoita H. 1989. Abnormal organization of keratin intermediate filaments in cultured keratinocytes of epidermolysis bullosa simplex. *Arch. Dermatol. Res.* 281:5–10

Kobori T, Montagna W. 1976. *Biology and Disease of the Hair.* Baltimore: Univ. Park Press. 667 pp.

Kopan R, Fuchs E. 1989. A new look into an old problem: keratins as tools to investigate determination, morphogenesis, and differentiation in skin. *Genes Dev.* 3:1–15

Kouklis P, Hutton E, Fuchs E. 1994. Making the connection: keratin intermediate filaments and desmosomes. *J. Cell Biol.* 127: 1049–60

Kremer H, Zeeuwen P, McLean WH, Mariman EC, Lane EB, et al. 1994. Ichthyosis bullosa of Siemens is caused by mutations in the keratin 2e gene. *J. Invest. Dermatol.* 103: 286–89

Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AHM, et al. 1992. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature* 356:244–46

Langbein L, Heid HW, Moll I, Franke WW. 1993. Molecular characterization of the body site-specific human epidermal cytokeratin 9: cDNA cloning, amino acid sequence, and tissue specificity of gene expression. *Differentiation* 55:57–71

Leask A, Rosenberg M, Vassar R, Fuchs E. 1990. Regulation of a human epidermal keratin gene: sequences and nuclear factors involved in keratinocyte-specific transcription. *Genes Dev.* 4:1985–98

Letai A, Coulombe P, Fuchs E. 1992. Do the ends justify the mean? Proline mutations at the ends of the keratin coiled-coil rod segment are more disruptive than internal mutations. *J. Cell Biol.* 116:1181–95

Letai A, Coulombe PA, McCormick MB, Yu QC, Hutton E, Fuchs E. 1993. Disease severity correlates with position of keratin point mutations in patients with epidermolysis bullosa simplex. *Proc. Natl. Acad. Sci. USA* 90:3197–201

Letai A, Fuchs E. 1995. The importance of intramolecular ion pairing in intermediate filaments. *Proc. Natl. Acad. Sci. USA* 92:92–96

Lloyd C, Yu Q-C, Cheng J, Turksen K, Degenstein L, et al. 1995. The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. *J. Cell Biol.* 129:1329–44

Loewinger L, McKeo F. 1988. Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. *EMBO J.* 7:2301–9

Lu B, Rothnagel JA, Langley MA, Tsai SY, Roop DR. 1994. Differentiation-specific expression of human keratin 1 is mediated by a composite AP-1/steroid hormone element. *J. Biol. Chem.* 269:7443–49

Lu X, Lane EB. 1990. Retrovirus-mediated transgenic keratin expression in cultured fibroblasts: specific domain functions in keratin stabilization and filament formation. *Cell* 62:681–96

Lynch MH, O'Guin WM, Hardy C, Mak L, Sun TT. 1986. Acidic and basic hair/nail ("hard") keratins—their colocalization in upper cortical and cuticle cells of the human hair follicle

and their relationship to "soft" keratins. *J. Cell Biol.* 103:2593–606
MacKinnon PJ, Powell BC, Rogers GE. 1990. Structure and expression of genes for a class of cysteine-rich proteins of the cuticle layers of differentiating wool and hair follicles. *J. Cell Biol.* 111:2587–600
Marchuk D, McCrohon S, Fuchs E. 1984. Remarkable conservation of structure among intermediate filament genes. *Cell* 39:491–98
Markova NG, Marekov LN, Chipev CC, Gan SQ, Idler WW, Steinert PM. 1993. Profilaggrin is a major epidermal calcium-binding protein. *Mol. Cell. Biol.* 13:613–25
Mathews CHE, Detmar K, Lawrence HJ, Largman C. 1993. Expression of the *hox 2.2* homeobox gene in murine embryonic epidermis. *Differentiation* 52:177–84
McCormick MB, Coulombe P, Fuchs E. 1991. Sorting out IF networks: consequences of domain swapping on IF recognition and assembly. *J. Cell Biol.* 113:1111–24
McLachlan AD. 1978. Coiled-coil formation and sequence regularities in the helical regions of α-keratins. *J. Mol. Biol.* 124:297–304
McLachlan AD, Stewart M. 1982. Periodic charge distribution in the intermediate filament proteins desmin and vimentin. *J. Mol. Biol.* 162:693–98
McLean WH, Morley SM, Lane EB, Eady RA, Griffiths WA, Paige DG, et al. 1994. Ichthyosis bullosa of Siemens—a disease involving keratin 2e. *J. Invest. Dermatol.* 103: 277–81
McLean WH, Rugg EL, Lunny DP, Morely SM, Lane EB, et al. 1995. Keratin 16 and keratin 17 mutations cause pachyonychia congenita. *Nature Genet.* 9:273–78
Mitchell PJ, Timmons PM, Hebert JM, Rigby PWJ, Tjian R. 1991. Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. *Genes Dev.* 5:105–19
Moll I, Heid HW, Franke WW, Moll R. 1988. Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. III. Hair and nail formation during human fetal development. *Differentiation* 39:167–84
Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors, and cultured cells. *Cell* 31: 11–24
Morasso JI, Jamrich M, Sargent TD. 1993. The homeodomain gene *Xenopus distal-less-like-2* (Xdll-s) is regulated by a conserved mechanism in amphibian and mammalian epidermis. *Dev. Biol.* 162:267–76
Nadeau JH, Berger FG, Cox DR, Crosby JL, Davisson MT, et al. 1989. A family of type I keratin genes and the homeobox-2 gene complex are closely linked to the *rex* locus on mouse chromosome 11. *Genomic* 5:454–62
Paller AS, Syder AJ, Chan YM, Yu QC, Hutton E, et al. 1994. A direct link between clinical and genetic mosaicism: the genetic basis of a form of epidermal nevus. *New Engl. J. Med.* 331:1408–15
Parry DAD, Crewther WG, Fraser RD, MacRae TP. 1977. Structure of α-keratin: structural implication of the amino acid sequence of the type I and type II chain segments. *J. Mol. Biol.* 113:449–54
Parry DAD, Steven AC, Steinert PM. 1985. The coiled-coil molecules of intermediate filaments consist of two parallel chains in exact axial register. *Biochem. Biophys. Res. Commun.* 127:1012–18
Pauling L, Corey RB. 1953. Compound helical configurations of polypeptide chains: structure of proteins of the α-keratin type. *Nature* 171:59–61
Powell B, Crocker L, Rogers G. 1992. Hair follicle differentiation: expression, structure and evolution of the hair type II keratin intermediate filament gene family. *Development* 114:417–33
Powell BC, Rogers GE. 1990. Cyclic hair-loss and regrowth in transgenic mice overexpressing an intermediate filament gene. *EMBO J.* 9:1485–93
Presland RB, Haydock PV, Fleckman P, Nirunsuksiri W, Dale BA. 1992. Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. *J. Biol. Chem.* 267:23772–81
Quinlan RA, Cohlberg JA, Schiller DL, Hatzfeld M, Franke WW. 1984. Heterotypic tetramer (A2D2) complexes of non-epidermal keratins isolated from cytoskeletons of rat hepatocytes and hepatoma cells. *J. Mol. Biol.* 178:365–88
Quinlan RA, Hatzfeld M, Franke WW, Lustig A, Schulthess T, Engel J. 1986. Characterization of dimer subunits of intermediate filament proteins. *J. Mol. Biol.* 192:337–49
Raats JMH, Henderik JBJ, Verdijk M, van Oort FLG, Gerards WLH, et al. 1991. Assembly of carboxy-terminally deleted desmin in vimentin-free cells. *Eur. J. Cell Biol.* 56:84–103
Raats JMH, Pieper FR, Egberts WTM, Verrijp KN, Ramaekers FCS, Bloemendal H. 1990. Assembly of amino terminally deleted desmin in vimentin-free cells. *J. Cell Biol.* 111: 1971–85
Redemann-Fibi B, Schuermann M, Muller R. 1991. Stage and tissue-specific expression of fosB during mouse development. *Differentiation* 46:43–49
Reis A, Hennies HC, Langbein L, Disweed M,

Mischke D, et al. 1994. Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK). *Nat. Genet.* 6:174–79

Rieger E, Bijl JJ, van Oostveen JW, Soyer HP, Oudejans CB, Jiwa NM, et al. 1994. Expression of the homeobox gene *HOXC4* in keratinocytes of normal skin and epithelial skin tumors is correlated with differentiation. *J. Invest. Dermatol.* 103:341–46

Rochat A, Kobayashi K, Barrandon Y. 1994. Location of stem cells of human hair follicles by clonal analysis. *Cell* 76:1063–73

Rogers GE, Powell BC. 1993. Organization and expression of hair follicle genes. *J. Invest. Dermatol.* 101:S50–S55

Rosenberg M, Fuchs E, Le Beau MM, Eddy R, Shows TB. 1991. Three epidermal and one epithelial keratin gene map to human chromosome 12. *Cell Cytogen.* 57:33–38

Rothnagel JA, Dominey AM, Dempsey LD, Longley MA, Greenhalgh DA, et al. 1992. Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. *Science* 257:1128–30

Rothnagel JA, Fisher MP, Axtell SM, Pittelkow MR, Anton-Lamprecht I, et al. 1993. A mutational hot spot in keratin 10 (KRT 10) in patients with epidermolytic hyperkeratosis. *Hum. Mol. Genet.* 2:2147–50

Rugg EL, McLean WHI, Lane EB, Pitera R, McMillan JR, et al. 1994. A functional "knockout" of human keratin 14. *Genes Dev.* 8:2563–73

Rugg EL, Morley SM, Smith FJD, Boxer M, Tidman MJ, et al. 1993. Missing links: Weber-Cockayne keratin mutations implicate the L12 linker domain in effective cytoskeleton function. *Nat. Genet.* 5:294–300

Ryynanen M, Knowlton RG, Uitto J. 1991. Mapping of epidermolysis bullosa simplex mutation to chromosome 12. *Am. J. Hum. Genet.* 49:978–84

Saitou M, Sugai S, Tanaka T, Shimouchi K, Fuchs E, et al. 1995. Immature skin development in transgenic mice with targeted expression of dominant-negative retinoic acid receptor. *Nature* 374:159–62

Smeyne RJ, Schilling K, Robertson L, Luk D, Oberdick J, et al. 1992. Fos-lacZ transgenic mice: mapping sites of gene induction in the central nervous system. *Neuron* 8:13–23

Snape AM, Jonas EA, Sargent TC. 1990. KTF-1, a transcriptional activator of *Xenopus* embryonic keratin expression. *Development* 109:157–65

Snape AM, Winning RS, Sargent TD. 1991. Transcription factor AP-2 is tissue-specific in *Xenopus* and closely related or identical to keratin transcription factor 1 (KTF-1). *Development* 113:283–93

Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI. 1981. Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. *Cell* 24:897–903

Stappenbeck TS, Green KJ. 1992. The desmoplakin carboxyl terminus coaligns with and specifically disrupts intermediate filament networks when expressed in cultured cells. *J. Cell Biol.* 116:1197–209

Stappenbeck, TS, Lamb JA, Corcoran CM, Green KJ. 1994. Phosphorylation of the desmoplakin COOH terminus negatively regulates its interaction with keratin intermediate filament networks. *J. Biol Chem.* 269: 29351–54

Steinert PM. 1990. The two-chain coiled-coil molecule of native epidermal keratin intermediate filaments is a type 1-type II heterodimer. *J. Biol. Chem.* 265:8766–74

Steinert PM, Marekov LN, Fraser RDB, Parry DAD. 1993. Keratin intermediate filament structure-crosslinking studies yield quantitative information on molecular dimensions and mechanisms of assembly. *J. Mol. Biol.* 230:436–52

Steinert PM, Parry DAD. 1993. The conserved H1 domain of the type II keratin 1 chain plays an essential role in the alignment of nearest neighbor molecules in mouse and human keratin 1/keratin 10 intermediate filaments at the two to four molecule level of structure. *J. Biol. Chem.* 268:2878–87

Steinert PM, Rice RH, Roop DR, Trus BL, Steven AC. 1983. Complete amino acid sequence of a mouse epidermal keratin subunit and implications for the structure of intermediate filaments. *Nature* 302:794–800

Stellmach V, Leask A, Fuchs E. 1991. Retinoid-mediated transcriptional regulation of keratin genes in human epidermal and squamous cell carcinoma cells. *Proc. Natl. Acad. Sci. USA* 88:4582–86

Stephens K, Sybert VP, Wijsman EM, Ehrlich P, Spencer A. 1993. A keratin 14 mutational hot spot for epidermolysis bullosa simplex, Dowling-Meara: implications for diagnosis. *J. Invest. Dermatol.* 101:240–43

Stewart M, Quinlan RA, Moir RD. 1989. Molecular interactions in paracrystals of a fragment corresponding to the α-helical coiled-coil rod portion of glial fibrillary acidic protein—evidence for an antiparallel packing of molecules and polymorphism related to intermediate filament structure. *J. Cell Biol.* 109:225–34

Sun TT, Eichner R, Schermer A, Cooper D, Nelson WG, Weiss RA. 1984. The transformed phenotype. In *The Cancer Cell,* ed. A Levine, W Topp, G van de Woude, JD Watson, 1:169–76. Cold Spring Harbor, NY: Cold Spring Harbor Lab.

Syder AJ, Yu QC, Paller AS, Giudice G, Pearson R, Fuchs E. 1994. Genetic mutations in

the *K1* and *K10* genes of patients with epidermolytic hyperkeratosis: correlation between location and disease severity. *J. Clin. Invest.* 93:1533–42

Takahashi K, Folmer J, Coulombe P. 1994. Increased expression of keratin 16 causes anomalies in cytoarchitecture and keratinization in transgenic mouse skin. *J. Cell Biol.* 127:505–20

Tanaka T, Parry DA, Klaus-Kovtun V, Steinert PM, Stanley JR. 1991. Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. *J. Biol. Chem.* 266:12555–59

Tomic-Canic M, Sunjevaric I, Freedberg IM, Blumenberg M. 1992. Identification of the retinoic acid and thyroid hormone receptor-responsive element in the human *K14* keratin gene. *J. Invest. Dermatol.* 99:842–47

Torchard D, Blanchet-Bardon C, Serova O, Langbein L, Narod S, et al. 1994. Epidermolytic palmoplantar keratoderma cosegregates with a keratin 9 mutation in a pedigree with breast and ovarian cancer. *Nat. Genet.* 6:106–9

Troyanovsky SM, Eshkind LG, Troyanovsky RB, Leube RE, Franke WW. 1993. Contributions of cytoplasmic domains of desmosomal cadherins to desmosome assembly and intermediate filament anchorage. *Cell* 72: 561–74

Tseng H, Green H. 1994. Association of basonuclin with ability of keratinocytes to multiply and with the absence of terminal differentiation. *J. Cell Biol.* 126:495–506

van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, et al. 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev.* 8: 2691–2703

Vassar R, Coulombe PA, Degenstein L, Albers K, Fuchs E. 1991. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* 64:365–80

Vassar R, Rosenberg M, Ross S, Tyner A, Fuchs E. 1989. Tissue-specific and differentiation-specific expression of a human *K14* keratin gene in transgenic mice. *Proc. Natl. Acad. Sci. USA* 86:1563–67

Viallet JP, Dhouailly D. 1994. Retinoic acid and mouse skin morphogenesis. 1. Expression pattern of retinoic acid receptor genes during hair vibrissa follicle, plantar, and nasal gland development. *J. Invest. Dermatol.* 103:116–21

Watt FM, Kubler D, Hotchin NA, Nicholson LJ, Adams JC. 1993. Regulation of keratinocyte terminal differentiation by integrin-extracellular matrix interactions. *J. Cell Sci.* 106:175–82

Weber K, Plessmann U, Dodemont H, Kossmagk-Stephan K. 1988. Amino acid sequences and homopolymer-forming ability of the intermediate filament proteins from an invertebrate epithelium. *EMBO J.* 7:2995–3001

Wiche G, Gromov D, Donovan A, Castanon MJ, Fuchs E. 1993. Expression of plectin mutant cDNA in cultured cells indicates a role of COOH-terminal domain in intermediate filament association. *J. Cell Biol.* 121: 607–19

Wilkinson DG, Bhatt S, Ryseck RP, Bravo R. 1989. Tissue-specific expression of c-jun and junB during organogenesis in the mouse. *Development* 106:465–71

Wong PC, Cleveland DW. 1990. Characterization of dominant and recessive assembly defective mutations in mouse neurofilament NF-M. *J. Cell Biol.* 111:1987–2003

Woods EF, Inglis AS. 1984. Organization of the coiled-coils in the wool microfibril. *Int. J. Biol. Macromol.* 6:277–83

Xu Z, Cork LC, Griffin JW, Cleveland DW. 1993. Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* 73:23–33

Zhou P, Byrne C, Jacobs J, Fuchs E. 1995. Lymphoid enhancer factor 1 (LEF1) plays a major role in hair follicle morphogenesis and gene expression. *Genes Dev.* 9:700–13