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Essay

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Of Mice and Men: Genetic Disorders of the Cytoskeleton

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Since the time when I was a postdoctoral fellow under the supervision of Dr. Howard Green, then at the Massachusetts Institute of Technology, I have been interested in understanding the molecular mechanisms underlying growth, differentiation, and development in the mammalian ectoderm. The ectoderm gives rise to epidermal keratinocytes and to neurons, which are the only two cell types of the body that devote most of their protein-synthesizing machinery to developing an elaborate cytoskeletal architecture composed of 10-nm intermediate filaments (IFs). Our interest is in understanding the architecture of the cytoskeleton in keratinocytes and in neurons, and in elucidating how perturbations in this architecture can lead to degenerative diseases of the skin and the nervous system. I will concentrate on the intermediate filament network of the skin and its associated genetic disorders, since this has been a long-standing interest of my laboratory at the University of Chicago.

KERATINS ARE THE MAJOR STRUCTURAL PROTEINS OF THE EPIDERMIS AND ITS APPENDAGES

At the interface between the physical traumas of our environment and our body is the epidermis, a stratified squamous epithelium, the outermost layer of which is the skin surface (Figure 1). The mitotically active cells of the epidermis are tucked safely away in the innermost, i.e., basal, layer; under an as yet unidentified trigger of terminal differentiation, a cell will withdraw from the cell cycle, commit to differentiate, and move outward toward the skin surface. In transit, the cell undergoes a variety of morphological and biochemical changes that culminate in the production of dead, flattened squames that are then shed from the skin surface, continually replaced by inner layer cells moving outward. Every 2–4 wk, there is a fresh epidermis to confront new traumas and to keep microorganisms out and essential bodily fluids in.

The epidermal cloak of armor serves an important protective role, which it accomplishes by producing an extensive cytoskeletal architecture, the unique feature of which is keratin filaments (for reviews, see Fuchs and Byrne, 1994; Fuchs, 1995). Keratins constitute more than 10% of the total protein of dividing keratinocytes and up to 85% of the total protein of fully differentiated cells. Although keratins are most abundant in the epidermis and its appendages, there are approximately 30 different keratin genes that are differentially expressed in all epithelial tissues at various stages of differentiation and development (Moll *et al.*, 1982). Keratins are often expressed as specific protein pairs, forming obligatory heteropolymers composed of stable heterodimers (Fuchs *et al.*, 1981; Moll *et al.*, 1982; Hanukoglu and Fuchs, 1983; Coulombe *et al.*, 1990; Hatzfeld and Weber, 1990a; Steinert, 1990). Keratins belong to the superfamily of intermediate filament (IF) proteins (for review, see Fuchs and Weber, 1994). In mammals, IFs impart to each specialized cell a cytoarchitecture tailored to suit its distinct needs. Much of what we know about 10-nm intermediate filament structure and assembly has been learned from studies on epidermal keratins.

There are four major keratin genes expressed in the epidermis. Mitotically active epidermal cells express keratin K5 and its partner K14 (Fuchs and Green, 1980; Moll *et al.*, 1982; Nelson and Sun, 1983). As keratinocytes withdraw from the cell cycle and commit to differentiate terminally, they switch off K5 and K14

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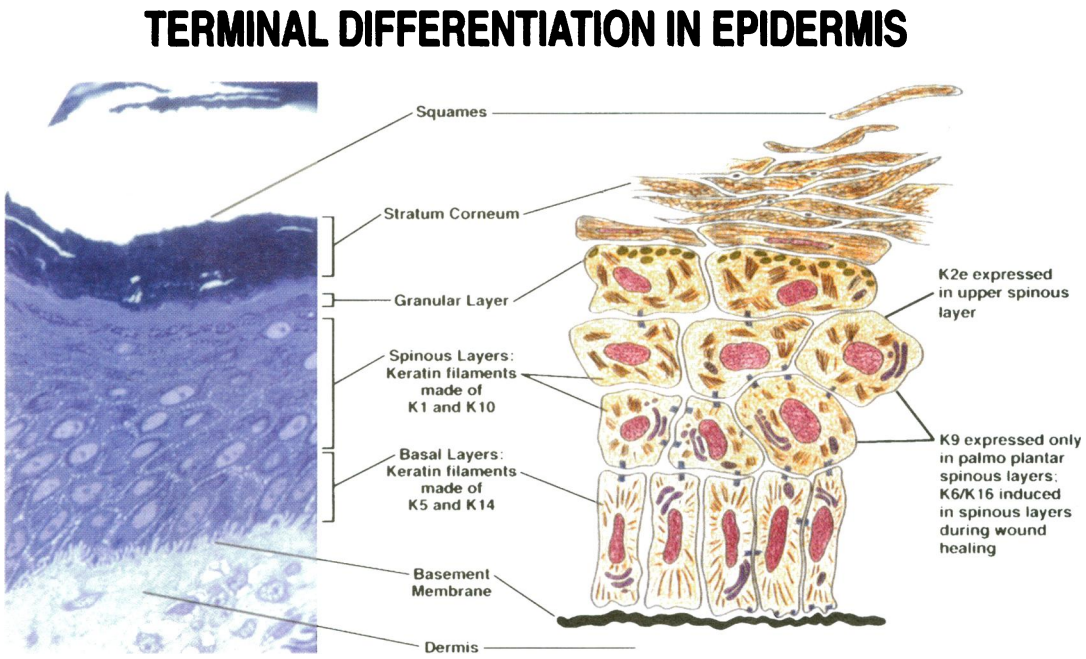


Figure 1. The epidermis and keratin expression. Epidermis: on the left is a cross-section of human skin stained with hematoxylin and eosin. The four major steps in epidermal differentiation are 1) an innermost basal layer of mitotically active cells; 2) three to six layers of spinous cells that are still transcriptionally active but are no longer dividing; these cells devote most of their translational machinery to expressing keratins; 3) one to three layers of granular cells that are transcriptionally active and deposit a cornified envelope of crosslinked proteins beneath the plasma membrane; and 4) 5–20 layers of stratum corneum, which consist of metabolically inert, enucleated squames that are sloughed from the skin surface. Basal epidermal cells express keratins 5 and 14. As basal cells commit to terminally differentiate, they switch off the expression of K5 and K14 and induce the expression of K1 and K10. As epidermal cells move up through the spinous layers, they express K2e, which can pair with K10. Some keratins are expressed in the epidermis under special circumstances: during wound healing, spinous cells induce the expression of keratins K6 and K16; K9 is unique to the suprabasal layers of palmar and plantar skin. Squames sloughed from the skin surface are merely dead sacs, chock full of keratin macrofibrils (permission to reproduce this figure granted from N. Engl. J. Med., Paller *et al.*, 1994).

and induce the expression of K1 and K10 (Fuchs and Green, 1980). Two additional partners for K10 are expressed in differentiating epidermal cells: K2e is expressed late in differentiation, appearing in the upper spinous layers of normal epidermis (Collin *et al.*, 1992); K9 is unique to the skin of the palms and the base of the foot and calluses (Fuchs and Green, 1980; Langbein *et al.*, 1994). During wound healing, a new set of keratins, K6 and K16, are induced in spinous cells; this keratin pair is also expressed in the outer root sheath of the hair follicle (Sun *et al.*, 1984; Mansbridge and Knapp, 1987; Paladini *et al.*, 1996). The cortex of the developing hair shaft differs completely from the epidermis in that a new set of keratins, the Ha and Hb keratins, are expressed (Moll *et al.*, 1982; Lynch *et al.*, 1986; Stark *et al.*, 1987; Coulombe *et al.*, 1989).

What is the functional significance of the complexity of keratins in the skin? While we do not yet know the underlying molecular explanation, it seems likely that the multiplicity of sequences enables the keratinocytes to meet different structural needs. For instance, mitotically active keratinocytes have keratin filaments that are relatively disperse in the cytoplasm, whereas K1 and K10 filaments of terminally differentiating cells form much thicker bundles of 10-nm filaments. In addition to variations in the extent to which keratin IFs associate with themselves, there are also differences in the associations that different keratin filaments make with other proteins in the cell. Thus, for example, keratin IFs attach to hemidesmosomes and desmosomes through specific sequences that are not conserved among all keratins (Stappenbeck and Green, 1992; Kouklis *et al.*, 1994). Moreover, as keratinocytes differentiate, they encounter newly synthesized IF-associated proteins such as filaggrin, a granular layer protein which promotes the bundling of keratin filaments into large macrofibrils (Dale *et al.*, 1978). Recently, it was shown that these macrofibrils associate with the cornified envelope through specific contacts that are unique to K1 and K10 (Steinert and Marekov, 1995). There are a plethora of specialized IF-associated proteins in hair cells, and although the exact nature of their interactions with IFs remains to be elucidated, they are likely to contribute substan-

Structure and Assembly of a Keratin Filament

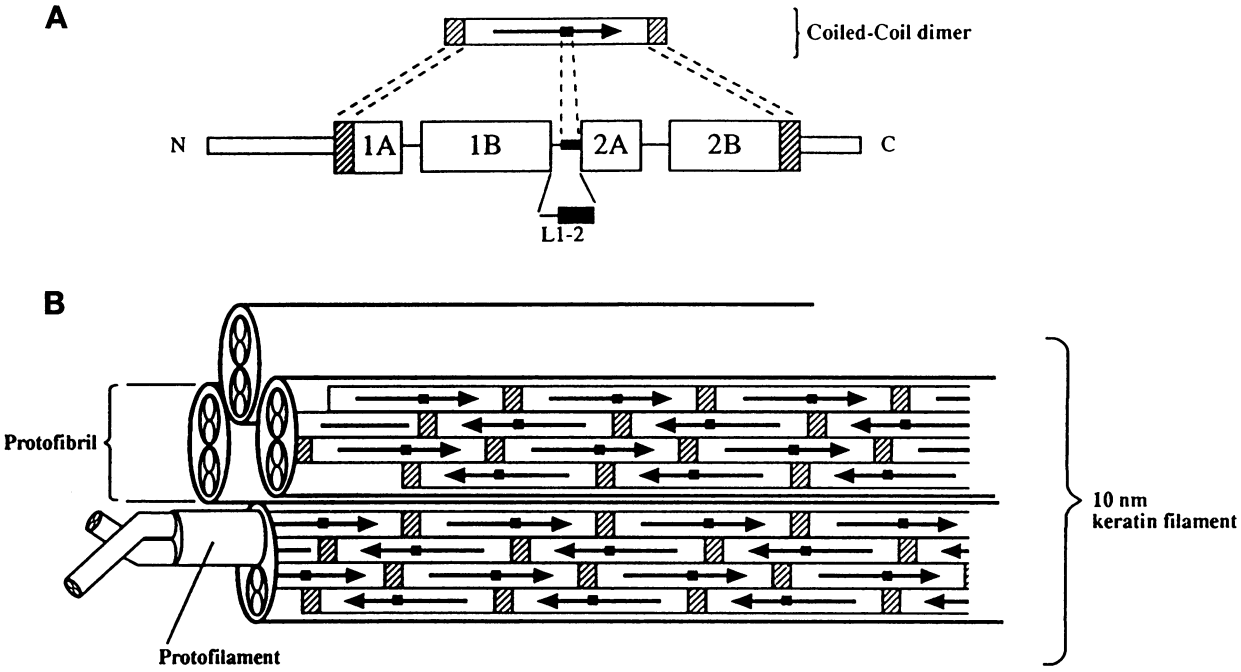


Figure 2. Model of keratin filament assembly. (A) Stick figure depicts the structure of a keratin heterodimer (Hanukoglu and Fuchs, 1982, 1983; Conway and Parry, 1988). Boxes denote the α-helical segments of the coiled coil rod; arrow indicates direction of polypeptides from base (N-terminus) to tip (C-terminus). Large boxes encompass the α-helical rod domain, interrupted by short nonhelical linker segments. Hatched boxes denote highly conserved ends of the rod. Thinner bars denote non-helical head and tail domains. Small black bar in each arrow denotes the sequence in the L1–2 linker region, which is relevant in W-C EBS mutations. (B) Putative arrangement of dimer subunits in the 10-nm keratin filament. Model is adapted from that previously described (Fuchs, 1994; permission granted from the J. Cell Biol.).

tially to the unique cytoarchitecture of the hair (Powell and Rogers, 1990).

KERATIN FILAMENTS: ASSEMBLY INTO 10-NM FIBERS

Inherent in the primary sequences of keratin proteins is the information to guide 10-nm filament assembly. In the electron microscope, keratin IFs appear as ropes of three or four strands called protofibrils (Aebi *et al.*, 1983; Steven *et al.*, 1983). Each protofibril is composed of two protofilaments, not easily visible by electron microscopy. Keratin protofilaments are thought to be composed of two antiparallel linear chains of IF protein dimers (Parry *et al.*, 1977; Aebi *et al.*, 1983, 1986; Conway and Parry, 1988). In each IF dimer, the two polypeptides are aligned in a parallel manner (Parry *et al.*, 1977), and dimer subunits are linked in a head to tail manner (Aebi *et al.*, 1986). Subunits in adjacent chains are thought to be half-staggered and antiparallel position relative to one another (Parry *et al.*, 1977; Conway and Parry, 1988; Geisler *et al.*, 1992; Steinert and Parry, 1993; Steinert *et al.*, 1993). Figure 2 illustrates the general features of 10-nm keratin filament structure.

All keratins have a central 310-amino acid residue "rod" flanked by non-helical amino "head" and carboxyl "tail" domains (Hanukoglu and Fuchs, 1982; 1983; Steinert *et al.*, 1983a). The rod contains sequences, known as heptad repeats of hydrophobic residues, that create one half of a zipper of hydrophobic residues that coil about the helical surface (Crick, 1953; Pauling and Corey, 1953). Two keratin polypeptides zip together by virtue of these hydrophobic interactions, thereby generating a coiled coil. Although conceptually convenient, the rod domain is not a continuous α-helix, but rather it is interspersed with three short linker segments that are predicted to perturb the α-helix (Figure 2; Hanukoglu and Fuchs, 1983; Conway and Parry, 1988). How these localized perturbations contribute to filament structure is unknown;

however, their conservation among all IF proteins tells us that they are important.

Despite remarkable structural similarities among the rod domains of different keratins and other IF proteins, their sequences are diverse. Keratin heterodimers consist of a member of each of two distinct sequence classes, which share only ~25–35% sequence identity within the rod segments. The sequences at the beginning and end of the rods are highly conserved even across subtypes. These rod ends are the most critical for the assembly of 10-nm keratin filaments in vivo and in vitro (Albers and Fuchs, 1987; 1989; Coulombe *et al.*, 1990; Hatzfeld and Weber, 1990b; Lu and Lane, 1990; Letai *et al.*, 1992; Wilson *et al.*, 1992), and even subtle point mutations in these segments can disrupt filament formation (Letai *et al.*, 1992). In contrast, proline mutations more centrally in the rod are often not as deleterious to the assembly process (Letai *et al.*, 1992).

In contrast to the α-helical rod domain, the nonhelical head and tail segments of keratins play less of a role in filament assembly and more of a specialized role. Among different pairs of keratins, end domains are hypervariable both in length and in sequence. Of the heads and tails of a keratin heteropolymer, only the type II keratin head domain appears to be critically involved in 10-nm filament assembly (Wilson *et al.*, 1992; Steinert and Parry, 1993). Intriguingly, the head and tail sequences within a keratin filament are more accessible to enzymatic reagents than the rod domain (Steinert *et al.*, 1983a). Thus, while the highly conserved rod segments are likely to impart a common structure to keratin filaments, the heads and tails are likely to participate in interkeratin filament associations and in the interaction of keratin filaments with other proteins in the cell.

EPIDERMOLYSIS BULLOSA SIMPLEX: DETERMINING THE PARADIGM FOR A GENETIC DISORDER OF KERATIN AND ELUCIDATING KERATIN FUNCTIONS

By 1985, it was apparent that keratins are the major structural proteins of the epidermis and its appendages, and that keratins are differentially expressed and encoded by different genes. This led us to wonder whether there might be genetic skin disorders that have as their basis defects in keratin genes in a manner perhaps analogous to blood disorders such as sickle cell anemia and thalassemias that have as their basis defects in globins, i.e., the major structural proteins of the blood. Classical human geneticists would select a disease and work their way toward the protein defect that causes the disease; my laboratory took an unconventional approach, choosing our protein and working our way toward the genetic diseases that might occur as a consequence of defects in keratin genes.

The molecular mutagenesis studies we conducted on the K5 and K14 pair of keratins led us to realize that most keratin mutations behave in a dominant negative manner, that is to say that they perturb filament assembly even in the presence of their wild-type partner keratin (Albers and Fuchs, 1987; 1989; Coulombe *et al.*, 1990; Letai *et al.*, 1992; Wilson *et al.*, 1992). We had used two assays in our approach: 1) transient transfection of mutant, epitope-tagged *K14* genes into cultured human epidermal cells (Albers and Fuchs, 1987); and 2) in vitro filament assembly assays with recombinant human K14 and K5 proteins (Coulombe *et al.*, 1990). Similar investigations by other groups studying additional keratins or other IF proteins strengthened our own conclusions, namely, that the amino and carboxyl ends of the α-helical rod domain are particularly critical for the assembly process. On the basis of these findings, we predicted that the majority of keratin disorders should display an autosomal dominant pattern of inheritance and that mutations or deletions in the conserved rod end domains should account for the most severe forms of the disorders.

But what human genetic diseases were likely to be keratin disorders? To address this question, we used transgenic mouse technology to target the expression of our mutant, epitope-tagged human *K14* genes to the skin (Coulombe *et al.*, 1991b; Vassar *et al.*, 1991). To drive expression, we used the human K14 promoter, which we had previously shown is faithfully active in the basal layer of the epidermis of transgenic mice (Vassar *et al.*, 1989). In the first set of experiments, transgenic mice expressing a carboxyl truncated, mutant human keratin 14 gene displayed the clinical and pathological features of the Dowling-Meara subtype of epidermolysis bullosa simplex (EBS) (Vassar *et al.*, 1991; Table 1). Of the three major subtypes of EBS, D-M EBS is the most severe and the rarest, affecting ~1/100,000 in the population. Clinical features are present at birth and are typified by mechanical stress-induced skin blistering due to cytolysis within the basal layer of the epidermis. Ultrastructurally, clumps or aggregates of keratin are present in the basal layer of D-M EBS epidermis (Figure 3A; Anton-Lamprecht,

Table 1. Characteristics of EBS

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

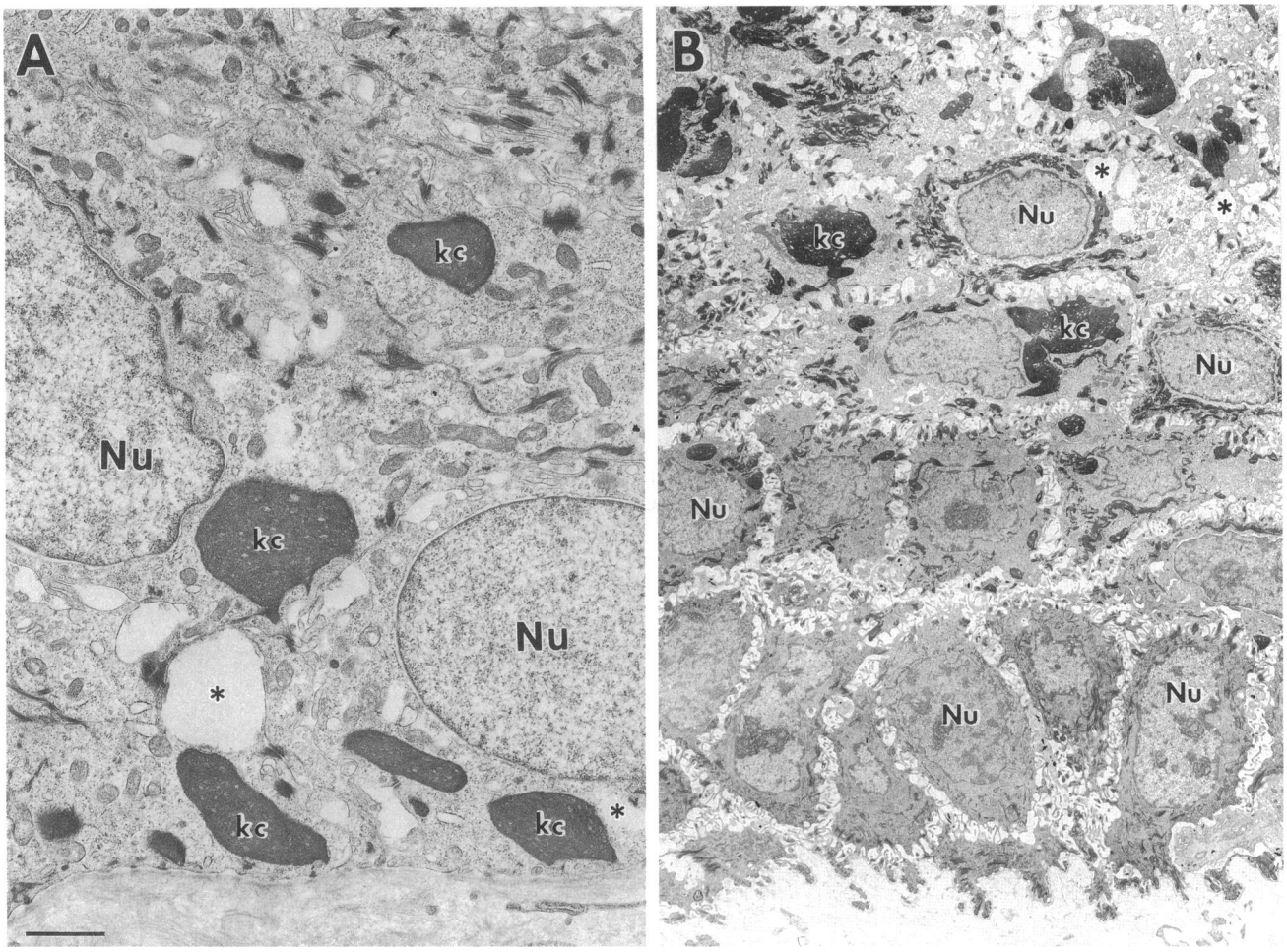


Figure 3. Ultrastructural characteristics of clinically severe EBS and EH. (A) Electron microscopy was performed on glutaraldehyde-fixed ultrathin sections of a skin biopsy from a patient with Dowling-Meara EBS (for method, see Cheng *et al.*, 1992). Note the presence of large clumps of amorphous keratin in the innermost basal cells of the epidermis. These clumps sometimes persist in the suprabasal layers, particularly in very severe cases such as the one shown here. Clumping of keratin often precedes overt signs of cytolysis and/or degeneration (asterisk). (B) Electron microscopy of ultrathin sections of a skin biopsy from a patient with severe EH. Note absence of keratin clumps in the basal layer, but presence of clumps and signs of cytolysis in the suprabasal layers. kc, keratin clumps; Nu, nucleus; asterisk, cytolysis/degeneration. Bars: A, 1 μm; B, 4 μm; electron microscopy by Dr. Q.-C. Yu.

1994 and references therein). All of these features were present in the K14 mutant-expressing mice, and, in addition, the basal clumps of keratin protein were shown to label with antibodies against the transgene and endogenous K14 and K5 proteins (Vassar *et al.*, 1991). In contrast, mice expressing more mildly disrupting K14 mutants exhibited features more typical of Weber-Cockayne EBS, with blistering primarily on their paws, and basal cell cytolysis, but with few aberrations in K5/K14 keratin filament networks (Coulombe *et al.*, 1991b).

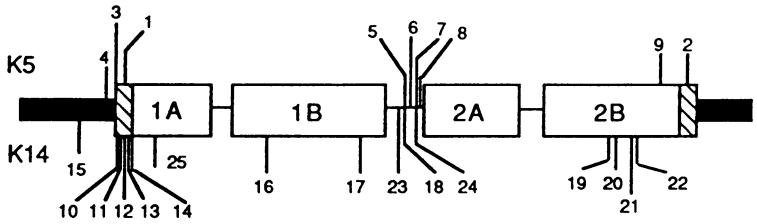
On the basis of the remarkable similarities between the epidermis of the transgenic mice and of human EBS patients, we postulated that EBS would be a disorder of keratins 14 and 5, and that the three major subtypes of EBS would be genetically related by virtue of where the mutations are located in the keratin polypeptides and the degree to which those mutations perturb the overall filament assembly process (Coulombe *et al.*, 1991b; Vassar *et al.*, 1991). Even though these predictions conflicted with biochemical and genetic mapping studies of the 1980s, they were consistent with early electron microscopy reports suggesting that perturbations in keratin filament networks are early events in the blistering process (Anton-Lamprecht and Schnyder, 1982). The parallels between cultured D-M EBS keratinocytes and keratinocytes transfected with mutant keratin genes had also been noted (Kitajima *et al.*, 1989), lending further support to this hypothesis.

Within 1 y after our first transgenic mouse report, it was discovered that humans with Dowling-Meara or

Summary of keratin mutations in EBS

K5 Mutation

No.	Subtype	Mutation	Domain
1.	D-M EBS	Δ30 aa	H1/1A conserved rod end
2.	D-M EBS	E475G	2B conserved rod end
3.	EBS-hom	K173N	H1/1A boundary
4.	W-C EBS	I161S (3)*	H1 non-helical head
4.	W-C EBS	I161N	H1 non-helical head
5.	W-C EBS	M327T	L1-2 non-helical linker
6.	W-C EBS	D328V	L1-2 non-helical linker
7.	W-C EBS	N329K	L1-2 non-helical linker
8.	W-C EBS	R331C	L1-2 non-helical linker
9.	K-EBS	L463P	2B helical rod



K14 Mutation

No.	Subtype	Mutation	Domain
10.	D-M EBS	M119I	1A conserved rod end
11.	D-M EBS	Q120R	1A conserved rod end
12.	D-M EBS	L122F	1A conserved rod end
13.	D-M EBS	R125C (6)	1A conserved rod end
13.	D-M EBS	R125H (9)	1A conserved rod end
13.	D-M EBS	R125S	1A conserved rod end
14.	D-M EBS	Y129D	1A conserved rod end
15.	K-EBS	G107stop	non-helical head
16.	K-EBS	Y204stop	1B helical rod
17.	K-EBS	A247D	1B helical rod
18.	K-EBS	M272R	L1-2 non-helical linker
19.	K-EBS	ΔE375	2B helical rod
20.	K-EBS	I377N	2B helical rod
21.	K-EBS	L384P	2B helical rod
22.	K-EBS	R388C	2B helical rod
23.	W-C EBS	V270M	L1-2 non-helical linker
24.	W-C EBS	A274D (3)	L1-2 non-helical linker
25.	EBS-rec	E144A	1A helical rod

* parentheses indicate # of distinct cases if greater than one.

Figure 4. EBS mutations relative to the secondary structure of keratin. Summary of mutations found in patients with EBS and correlation between the location of mutation and disease severity of EBS. Structure of keratins is as described in the legend to Figure 2. D-M, K, and W-C EBS mutations are those identified in the literature to date. Note that D-M EBS mutations cluster within the highly conserved ends, K-EBS mutations are within the α-helical segments but are more internal, and W-C EBS mutations are in the nonhelical regions of keratin, particularly in the L1–2 linker segment (reprinted with permission from Blackwell Publishers).

Koebner EBS have point mutations in their *K14* or *K5* genes (Bonifas *et al.*, 1991; Coulombe *et al.*, 1991a; Lane *et al.*, 1992), and that these defects reside at chromosomes 17q12–21 and 12q11–12, i.e., at the loci of the type I and type II keratin gene clusters, respectively (Rosenberg *et al.*, 1988; 1991; Bonifas *et al.*, 1991; Chan *et al.*, 1993; 1994b). Figure 4 summarizes the locations of these mutations and their clinical severity. The arginine residue at position 125 (R125) in the K14 polypeptide is a hot spot for mutagenesis to either histidine or cysteine (R125H/C) and accounts for the majority of D-M EBS cases analyzed thus far (Coulombe *et al.*, 1991a; Stephens *et al.*, 1993; Chen *et al.*, 1995; Chan *et al.*, 1996). Located within the highly conserved amino end of the K14 rod domain, the arginine codon (CGC) appears to be both a target for methylation and subsequent deamination in the epidermis and also a residue that is essential for filament assembly.

Although D-M EBS cases tend to have mutations within the highly conserved ends of the rod domain of K5 or K14 (Coulombe *et al.*, 1991a; Lane *et al.*, 1992; Hovnanian *et al.*, 1993; Stephens *et al.*, 1993; Chen *et al.*, 1995; Chan *et al.*, 1996), K EBS cases are

frequently proline residues and are located more centrally within the α-helical rod segments (Bonifas *et al.*, 1991; Dong *et al.*, 1993; Humphries *et al.*, 1993; Yamanishi *et al.*, 1994). Interestingly, in filament assembly and/or gene transfection assays, the Dowling-Meara EBS mutations all produce short filament rodlets, suggesting that the process of filament elongation is compromised (Coulombe *et al.*, 1991a; Letai *et al.*, 1993; Chan *et al.*, 1994). In contrast, Koebner mutations are less severe, producing somewhat longer filaments (Letai *et al.*, 1993).

Weber-Cockayne EBS is also a disorder of K5 and K14. This was first suggested by genetic mapping analysis (Bonifas *et al.*, 1991) and later demonstrated by identifying specific mutations in affected family members (Chan *et al.*, 1993; 1994b; Rugg *et al.*, 1993). In contrast to D-M and K EBS, most of the W-C mutations are located in the nonhelical segments of the keratin polypeptide, and many of the mutations are in K5 rather than K14. Many of the W-C EBS mutations identified thus far reside in the nonhelical linker segment (L1–2) that separates helix 1B from helix 2A (Rugg *et al.*, 1993; Chan *et al.*, 1994b; Figure 4). Interestingly, when combined with their wild-type partner keratin, these W-C EBS mutations produce filaments which are often unraveled, suggesting that the L1–2 residues may be involved in the lateral positioning or the association of the linear arrays of dimers that exist within a 10-nm filament (Chan *et al.*, 1994b).

Collectively, our filament assembly studies on the Dowling-Meara, Koebner and Weber-Cockayne mutations suggest that the end domains of the rod are especially critical for filament elongation, whereas the central linker segment plays a role in lateral packing of subunits. Additionally, our findings imply that filament length is more critical to mechanical strength than lateral associations between protofibrils and/or protofilaments. Finally, these data reveal a clear relationship between the severity of the disease, the location of the mutation, and the degree to which a K5/K14 mutation affects 10-nm filament assembly.

Can an understanding of keratin filament assembly and EBS also tell us about the function(s) of keratin filaments specifically and IFs more generally? A clue to keratin function comes from the fact that the basal epidermal layer undergoes cytolysis in response to mechanical stress. A priori, it could be argued that clumps or aggregates of keratin (or any other protein) might compromise the physiology of the keratinocyte and lead to release of proteases and cell destruction. Alternatively, the cells might be ruptured because the keratin cytoarchitecture is perturbed. Analysis of several rare autosomal recessive cases of EBS provided clues that the keratin network is the key to the process (Chan *et al.*, 1994a; Rugg *et al.*, 1994; Jonkman *et al.*, 1996). Generation of mice completely null for K14 confirmed this notion (Lloyd *et al.*, 1995). We already knew that

Table 2. Disorders of intermediate filaments and their cytoskeletal networks

Disorder	Cells involved	Species	Genes mutated
EBS	Basal epidermal	Mouse/human	*K5, K14*
Dowling-Meara EBS			
Koebner EBS			
Weber-Cockayne EBS			
EBS w/mottled pigmentation	Basal epidermal	Human	*K5*
EBS w/muscular dystrophy	Basal epidermal/muscle	Human	*plectin*
EBS w/sensory neuron degeneration	Basal epidermal/DRG	Mouse	*BPAG1*
Junctional epidermolysis bullosa	Epidermal/Derm. Jnct.	Mouse/human	*α6β4, laminin V*
EH	Suprabasal epidermal	Mouse/human	*K1, K10*
Ichthyosis bullosa of siemens (mild EH)	Upper suprabasal	Human	*K10, K2e*
Epidermal nevi/EH type (mosaic EH)	Suprabasal epidermal/mosaic	Human	*K1, K10*
Epidermolytic palmoplantar keratoderma	Suprabasal palmoplantar	Human	*K9*[a]
Pachyonychia congenita	Nails, hair, epidermis near follicle openings	Mouse/human	*K6, K16, K17*[b]
White sponge nevus	Oral epithelia Esophagus	Human	*K4, K13*
Chronic hepatitis	Liver	Mouse	*K18*
Motor neuron disease	Motor neurons	Mouse	*NFL*[c]

[a] Torchard *et al.*, 1994; Reis *et al.*, 1994.

[b] McLean *et al.*, 1995; Bowden *et al.*, 1995.

[c] Xu *et al.*, 1993; Cote *et al.*, 1993.

in the absence of K14, K5 is unstable and turns over rapidly (Lersch and Fuchs, unpublished data); now, with skin blistering still present in the absence of a basal K5/K14 network, it was clear that the function of this network is to impart mechanical integrity to the basal keratinocyte, without which the cell becomes fragile and prone to rupture upon physical trauma.

Additional insights to keratin function have surfaced in exploring patients with EBS and mottled pigmentation. Patients with this disorder appear to have perturbations in the distribution of melanosomes within their epidermal basal cells. It was surprising to find that affected members of two apparently unrelated families with this disorder have the exact same point substitution, a proline to a leucine at residue 24, in the nonhelical head domain of K5 (Uttam *et al.*, 1996). Given that EBS can be caused by so many different mutations, this makes it unlikely that a second mutation is responsible for the mottled pigmentation phenotype. The P24L substitution is intriguing in that 1) it is within a region likely to protrude along the filament surface (Steinert *et al.*, 1983a); 2) it is predicted to cause a significant conformational change in the K5 head domain; and 3) the K5 head domain associates with desmoplakin, a desmosomal protein (Stappenbeck and Green, 1992; Kouklis *et al.*, 1994). Whether this change alters the transfer of melanin granules from the melanocyte to the keratinocyte, or whether it perturbs the normal positioning of the protective melanin granules over the nucleus of the mitotically active basal keratinocyte remains to be explored. However, it is interesting that while long regarded as strictly a microtubule-driven process, melanosome distribution has recently been shown to be aberrant in the myosin V null mouse (Mercer *et al.*, 1991), and now a keratin mutation may affect the process as well. One possible explanation is that connections exist between different cytoskeletal components and that by perturbing one network, an effect on another filament system occurs.

A GROWING LIST OF KERATIN DISORDERS AND THE POTENTIAL FOR OTHER IF DISORDERS

Once the paradigm was determined for one keratin disorder, it was possible to readily predict which other skin diseases might be disorders of keratin. Keratin disorders would be expected to display 1) clumping or perturbations in the keratin IF network; and 2) cell cytolysis, often associated with mechanical stress. Given that the patterns of keratin expression are well known (Moll *et al.*, 1982), a search for mutations can then be made in the appropriate keratin pair. A disease that fits the paradigm for a keratin disorder is epidermolytic hyperkeratosis. In this epidermal disorder, the clinical and ultrastructural features of the spinous layers are a mirror image of those seen in the basal layer of EBS epidermis (Figure 3B; Anton-Lamprecht, 1994), suggesting the likelihood that EH might be a disorder of K1 and K10 (Vassar *et al.*, 1991). Soon after, it was shown that transgenic mice expressing a mutant *K10* gene display the characteristic features of EH (Fuchs *et al.*, 1992). Genetic mapping data (Compton *et al.*, 1992) and sequence analyses (Cheng *et al.*, 1992; Chipev *et al.*, 1992; Rothnagel *et al.*, 1992) indicated that affected members of families with EH have point mutations in their *K1* or *K10* genes. Similar to EBS, many of the most severe cases of EH display mutations in the highly conserved ends of the rod domain, and, in some cases, e.g., the highly conserved and frequently mutated arginine residue discussed above, this mutation is also found in *K10*, where it is a hot spot for EH (Cheng *et al.*, 1992; Rothnagel *et al.*, 1992). Mutations in the *K2e* gene also occur, and consistent with the late onset of *K2e* expression (Collin *et al.*, 1992), these patients exhibit only mild clinical features even when the mutated residue is in a highly conserved rod end (Kremer *et al.*, 1994; McLean *et al.*, 1994; Rothnagel *et al.*, 1994). An additional mild case, often referred to as the Siemens subtype, has also been found to harbor a *K10* mutation, but in this case, the mutation falls outside the highly conserved end domains of the rod segment (Syder *et al.*, 1994).

Interestingly, when patients acquire a post-zygotic mutation in their *K10* gene, they display stripes or lines of EH on their body surface (Paller *et al.*, 1994). Previously referred to as a form of epidermal nevus (EN), mosaic EH has no counterpart in EBS. The most likely reason for this is that in a genetically mosaic disorder of mitotically active keratinocytes, wild-type cells in the basal layer can move laterally to fill a vacancy left by a degenerating mutant cell. Consequently, in a situation where 50% or more of the basal cells are wild type, the epidermis will quickly be taken over by the wild-type cells, leaving a diagnosis of clinically normal. In contrast, in a genetically mosaic disorder of differentiating keratinocytes, cells are already locked into a columnar upward movement by the time they first exhibit clinical signs of the disorder. In this case, no compensation can occur since wild-type spinous cells cannot move laterally. Thus, a diagnosis of epidermal nevi of the EH type is made.

The tree of keratin disorders continues to branch and now includes nonepidermal diseases such as white sponge nevus, an esophageal and oral epithelial disorder in humans (Richard *et al.*, 1995; Rugg *et al.*, 1995), and chronic hepatitis in mice (Ku *et al.*, 1995). Table 2 outlines those that have been verified genetically. However, other disorders fit the paradigm even

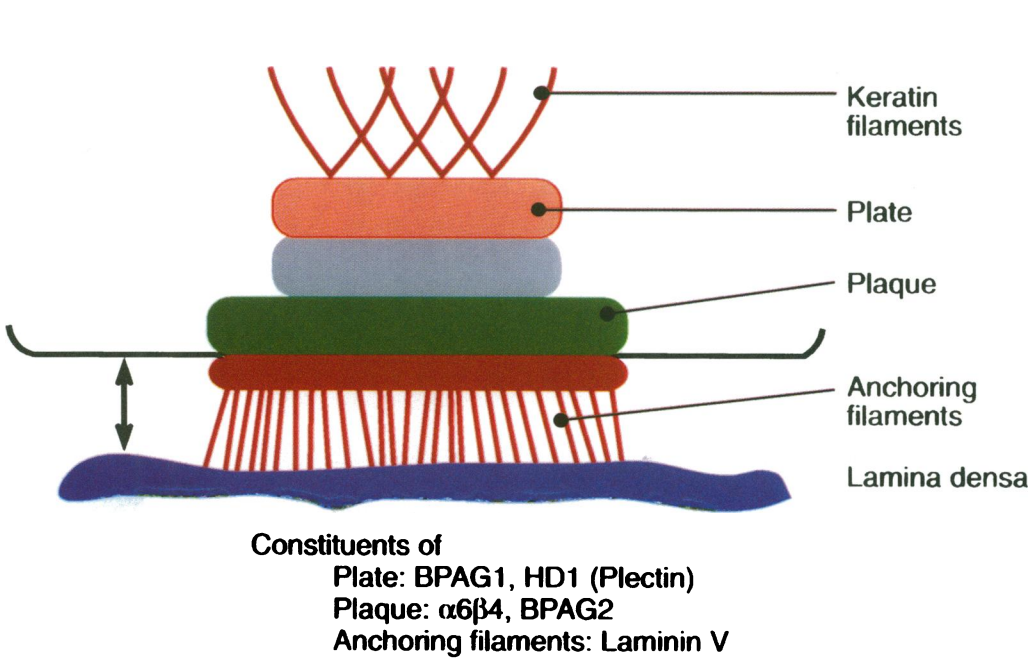


Figure 5. Structure of a hemidesmosome. Hemidesmosomes are characteristic of all stratified squamous epithelia. They are electron-dense membrane plaques at the base of the innermost basal layer. On their exterior, they attach to anchoring filaments composed of laminin V. On their interior, they attach to keratin filaments. There are four major components of hemidesmosomes: $\alpha 6\beta 4$ integrin, BPAG1-e, BPAG2 (collagen XVII), and HD1 (plectin).

though mutations in patients have not yet been identified. Thus, given that there are more than 30 different keratin genes in the human genome, it is likely that this list will continue to grow. Additional keratin-based diseases are likely to encompass hair disorders involving brittleness and degeneration. Abnormalities in two-dimensional gel patterns and amino acid compositions of hair keratins from patients with hair diseases have been reported (see Gillespie and Marshall, 1989). In addition, a number of autosomal dominant mouse mutants, Re, Bsk, and Re^{den}, map in close proximity to the type I epidermal keratin genes and have abnormalities in their hairs (Nadeau *et al.*, 1989 and references therein). Recently, the human hair disorder monilethrix has been linked to the keratin gene cluster on chromosome 12q11-q13 (Healy *et al.*, 1995; Stevens *et al.*, 1996).

Finally, it is interesting that there are more than 50 different IF genes in the human genome, and virtually every cell in the body contains some type of intermediate filament network. In this regard, certain types of neurodegenerative disorders and familial cardiomyopathies fit the paradigm for an IF disorder. Although such human disorders have not yet been identified at a genetic level, it seems likely that it is just a matter of time before these or other degenerative diseases are included in the group of IF diseases.

CYTOARCHITECTURE AND THE FUNCTIONAL SIGNIFICANCE OF PLASMA MEMBRANE ATTACHMENTS TO INTERMEDIATE FILAMENTS: DISCOVERING GENETIC DISORDERS OF THE CYTOSKELETON THAT GO BEYOND THOSE OF THE SKIN

A keratinocyte is not simply a bag of keratin filaments. Rather, keratins spin an intricate web of filaments that stretch out across a cell, extending from the nuclear envelope in the center of the cell to the desmosomes and hemidesmosomes at the cell periphery. While exploring the biochemical nature and the functional significance of the attachments of IFs to the plasma membrane, our search has made an unexpected turn, leading us beyond the epidermis and into the nervous system.

All stratified squamous epithelia including the epidermis contain numerous hemidesmosomes, which are electron-dense membrane plaques located at the base of the basal epithelial layer (Figure 5). Filaments composed of laminin V anchor hemidesmosomes to the underlying basement membrane of the extracellular matrix. At the core of the hemidesmosome is $\alpha 6\beta 4$ integrin, unusual in that it attaches to the keratin filament network rather than the actin microfilaments (for review, see Garrod, 1993). Two

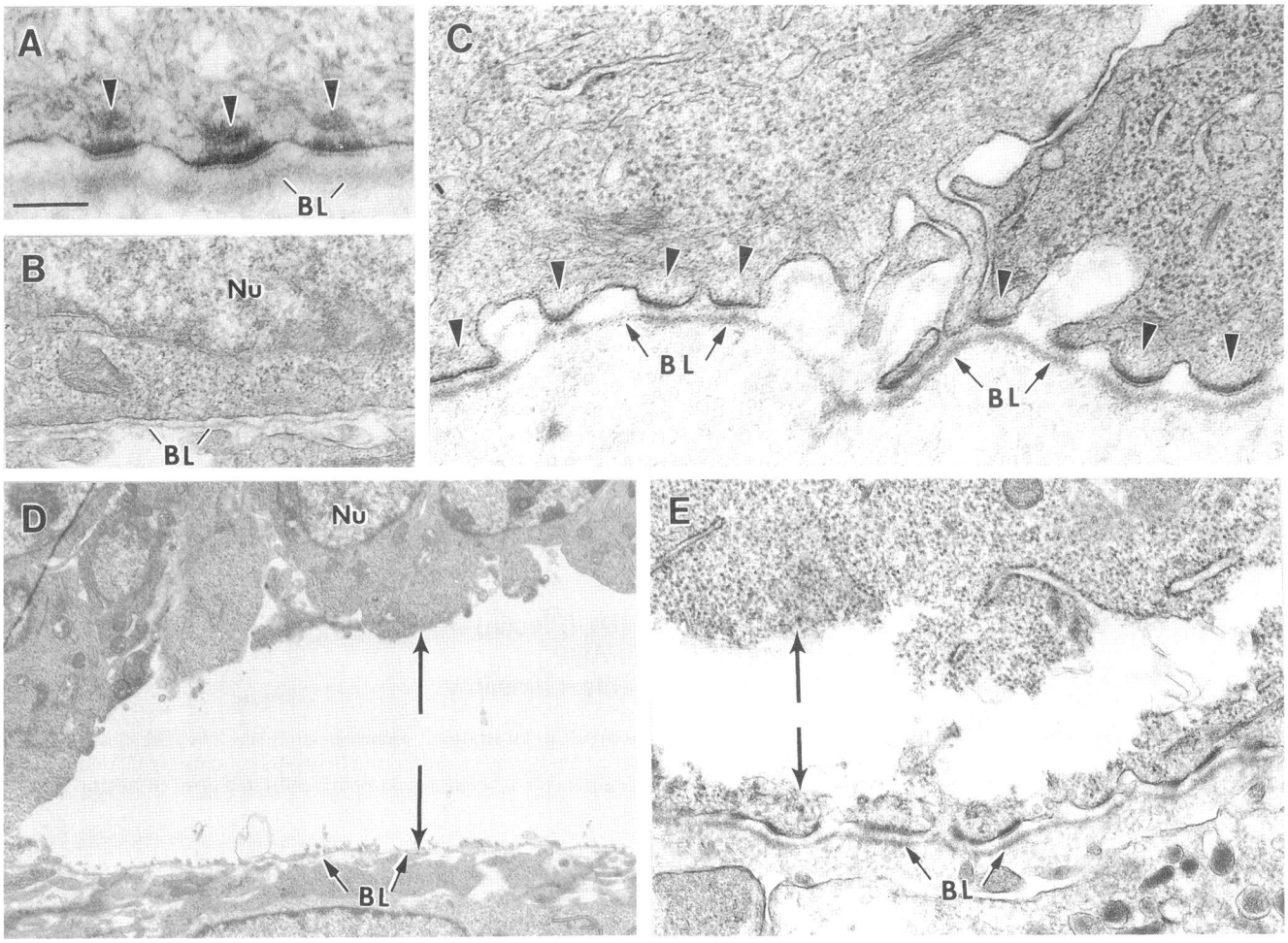


Figure 6. Ultrastructure of hemidesmosomes in normal epidermis and in epidermis from mice targeted for either the β4 integrin or the *BPAG1* gene. Fixation and processing of tissues were as described in Guo *et al.* (1995). Ultrathin sections are from the skins of either control mice (A), β4 knockout mice (B and D), or BPAG1 knockout mice (C and E). Depicted here are normal hemidesmosomes (A), illustrating the presence of the inner plate and the attachment to keratin filaments; lack of hemidesmosomes in the β4 knockout mouse (B); presence of hemidesmosomal plaques that are missing the inner plate and that lack keratin filaments (C); Note: cell on the left in (C) is in metaphase; split in β4 knockout skin at the epidermal-dermal junction (D); split in the BPAG1 knockout skin within the basal epidermal cells, just above the hemidesmosomes (E). Arrowheads, inner plate in (A) or lack thereof in (C); double arrows, blisters. BL, basal lamina; Nu, nucleus. Bars: A, 0.2 μm; B, 0.5 μm; C, 0.3 μm; D, 2.3 μm; E, 0.4.

additional proteins, BPAG1-e and BPAG2, impart to the hemidesmosome its distinctive structure. BPAG1-e is a protein that resides at the inner plate of the hemidesmosome, where the keratin filaments seem to thread through the inner surface of the hemidesmosome; BPAG2 is a transmembrane protein with an extracellular domain that places it in the collagen family. These unusual proteins received their name from the fact that patients with the autoimmune disease, bullous pemphigoid, produce autoantisera against these proteins (for review, see Stanley, 1993).

What is the function of the hemidesmosome? Recently, we used gene-targeting technology to ablate β4 integrin in mice (Dowling *et al.*, 1996; Georges-Labouesse *et al.*, 1996; van der Neut *et al.*, 1996). In the absence of β4, α6 is unstable, leading to a complete loss of hemidesmosomes in these animals. The mice develop clinical signs of a devastating human blistering disorder referred to as junctional epidermolysis bullosa. Patients with this disorder have been found to have premature stop codons or small internal deletions in either their laminin V chains or their β4 gene (see Vidal *et al.*, 1995; Christiano and Uitto, 1996). In our null mice, the complete loss of hemidesmosomes leads to a drastic weakening of cell-substratum contacts (Figure 6,B and D; Dowling *et al.*, 1996). These functions seem to be distinct from those of α3β1 integrins, which even though present in basal epidermal cells, do not seem able to compensate for the loss of α6β4.

What is the function of the attachment of keratin filaments to the hemidesmosome? To answer this question, we used gene targeting to ablate the *BPAG1* gene in mice (Guo *et al.*, 1995). Removing the coiled coil BPAG1-e protein from the hemidesmosome severs the connection between the hemidesmosome and its keratin filament network (Figure 6C; Guo *et al.*, 1995). Does this weaken cell-substratum adhesion or alternatively does it affect mechanical integrity? When the skin of the BPAG1 null mice is subjected to mild physical trauma, the answer is readily apparent: the epidermis tears just above its base, leaving a sheet of basement membrane-attached hemidesmosomes behind (Figure 6E; Guo *et al.*, 1995).

Our results argue that the function of the attachment of keratin filaments to hemidesmosomes is a structural one and not one that dramatically affects cell adhesion. Thus, defects in BPAG1e in humans are likely to give rise to EBS, but a form of EBS which is likely to be milder than that caused by severely disrupting keratin 5 or 14. Other hemidesmosomal proteins, perhaps HD1 (plectin), that might be involved in keratin filament attachment would also be predicted to give rise to EBS when defective in humans.

Given what we now know about hemidesmosomal defects, what can we postulate about putative desmosomal mutations in mice or in humans? Desmosomes are adherens junctions composed of cadherins (desmogleins and desmocollins) rather than integrins (for review, see Garrod, 1993). In transgenic mice, a dominant negative desmoglein perturbs desmosomal structure and gives rise to a thickened, hyperkeratotic skin and to hair defects (Allen *et al.*, 1996). The inner surface of desmosomes interfaces with keratin filaments, and although BPAG1e is not involved in this connection, there are two BPAG1e-related proteins that localize here: desmoplakin (Garrod, 1993) and the recently identified envoplakin (Ruhrberg *et al.*, 1996). Extrapolating from our findings on BPAG1-e and considering that desmosomes are present at all cell–cell borders of basal and suprabasal cells, we speculate that defects in desmoplakin or envoplakin might give rise to quite severe mechanical perturbations. In the skin, such alterations might be expected to be similar to EBS but extend into the upper layers. In this regard, it is interesting that a nonepidermolytic case of palmoplantar keratoderma was recently shown to arise from a mutation in the head domain of K1 (Kimonis *et al.*, 1994) at a lysine residue that has been shown to be involved in the association of basal epidermal keratin filaments with desmoplakin (Kouklis *et al.*, 1994). Given that desmoplakin is expressed in many tissues including muscle (for review, see Garrod, 1993), defects in desmoplakin might have deleterious consequences that extend beyond the skin; however, envoplakin appears to be more restricted in its expression (Ruhrberg *et al.*, 1996), and, when mutated, might be expected to give rise to perturbations in the suprabasal layers of the epidermis. Future studies should determine the extent to which these predictions are valid.

In contrast to desmoplakin, the expression of BPAG1-e was thought to be restricted largely to stratified epithelia (Owaribe *et al.*, 1990). Thus, we were particularly surprised when 2 wk after the birth of our BPAG1 null mice, the animals began to adopt peculiar postures, coupled with dystonic, spastic paraplegia (Guo *et al.*, 1995). A thorough analysis of these animals led us to realize that we had inadvertently discovered the genetic basis of a well-known neurological mouse mutant, of unknown etiology, referred to as *dystonia musculorum* (Guo *et al.*, 1995). Soon afterward this finding was confirmed by Brown *et al.* (1995), who came to the same conclusion using positional cloning to identify the *dt* locus. The classical feature of these mice is that they display gross and rapid degeneration of their sensory nervous system, leaving the animals crippled by 5 wk of age.

How is BPAG1 related to sensory neuron disease? Both groups have cloned neuronal forms of the *BPAG1* gene. The complete BPAG1-n form reveals the presence of an actin-binding domain at the unique amino end of the coiled coil rod segment and an intermediate filament binding domain, shared by BPAG1-e, at the other end (Yang *et al.*, 1996). Intriguingly, this novel protein has the capacity to bind to both cytoskeletal networks simultaneously (Yang *et al.*, 1996). In sensory neurons, the protein localizes to the axon, where it could function by anchoring the neurofilament cytoskeleton to the plasma-membrane associated actin network (Yang *et al.*, 1996).

The discovery of a protein that can link the actin cytoskeleton to the intermediate filament cytoskeleton is exciting, given that previously such associations have been largely speculative. Moreover, the dramatic decline of the sensory nervous system in the BPAG1 null mice argues that this interaction is critical to the survival of at least some neurons. Do other cell types have such connections, and are they important for cell survival? Interestingly, a related protein called plectin also has a putative actin-binding domain in its amino-terminal domain (Yang *et al.*, 1996) and a bona fide IF-associated domain in its carboxyl-terminal segment (Wiche *et al.*, 1993). Recently, it was shown that defects in plectin give rise to patients with the combined disorder of EBS and muscular dystrophy (Gache *et al.*, 1996; McLean *et al.*, 1996; Smith *et al.*, 1996). Plectin is expressed in muscle and in skin, and in the epidermis, plectin associates with hemidesmosomes (Gache *et al.*, 1996).

Although the group of proteins sharing sequence homology with BPAG1-e is small, it is growing. This class of proteins appears to have the unique properties to associate with the IF cytoskeleton at the carboxyl end of their α-helical rod segment, leaving the amino

end free to anchor the IF network to various locations within the cell. In the future, this group of interesting proteins is likely to provide us with further insights into cytoarchitecture and into how perturbations in cytoarchitecture lead to human disease.

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