A Function for Keratins and a Common Thread among Different Types of Epidermolysis Bullosa Simplex Diseases

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Abstract. Previously we demonstrated that transgenic mice expressing a mutant keratin in the basal layer of their stratified squamous epithelia exhibited a phenotype bearing resemblance to a subclass (Dowling Meara) of a heterogeneous group of human skin disorders known as epidermolysis bullosa simplex (EBS) (Vassar, R., P. A. Coulombe, L. Degenstein, K. Albers, E. Fuchs. 1991. *Cell.* 64:365–380.). The extent to which subtypes of EBS diseases might be genetically related is unknown, although they all exhibit skin blistering as a consequence of basal cell cytolysis. We have now examined transgenic mice expressing a range of keratin mutants which perturb keratin filament assembly to varying degrees. We have generated phenotypes which include most subtypes of EBS, demonstrating for the first time that at least in mice, these diseases can be generated by different mutations within a single gene. A strong correlation existed between the severity of the disease and the extent to which the keratin filament network was disrupted, implicating perturbations in keratin networks as an essential component of these diseases. Some keratin mutants elicited subtle perturbations, with no signs of the tonofilament clumping typical of Dowling-Meara EBS and our previous transgenic mice. Importantly, basal cell cytolysis still occurred, thereby uncoupling cytolysis from the generation of large, insoluble cytoplasmic protein aggregates. Moreover, cell rupture occurred in a narrowly defined subnuclear zone, and seemed to involve three factors: (*a*) filament perturbation, (*b*) the columnar shape of the basal cell, and (*c*) physical trauma. This work provides the best evidence to date for a structural function of a cytoplasmic intermediate filament network, namely to impart mechanical integrity to the cell in the context of its tissue.

THE epidermis provides the protective interface between various chemical and physical traumas of the environment and the rest of the bodily organs. It manifests its protective function by building an extensive cytoskeletal network of 10-nm intermediate filaments (IFs)[1] composed of a 1:1 ratio of type I and type II keratins. During development, embryonic basal cells are the first to express detectable levels of the type I keratin K14 (50 kD) and type II keratin K5 (58 kD) (Jackson et al., 1981; Dale et al., 1985). Their expression is then elevated greatly at a time when stratification and commitment to an epidermal cell fate take place (Kopan and Fuchs, 1989). In rodents, this occurs shortly before birth, whereas in humans, this occurs after the first trimester in utero. As basal epidermal cells differentiate, they downregulate K5/K14 expression and switch on a new pair of keratins, K1 (67-kD) and K10 (56.5-kD) (Fuchs and Green, 1980; Roop et al., 1987). In the fully differentiated squame, these keratins constitute ~85% of the total cellular protein.

Given the abundance of keratin in epidermis, it seems surprising that the function of these proteins has remained elusive. Since they are among the few proteins left in terminally differentiated squames, it has been assumed that one function of these keratins is to survive, a process which may be accomplished by (*a*) the extraordinary stability of their interprotein interactions (Franke et al., 1983; Coulombe and Fuchs, 1990); and (*b*) the progressive bundling of keratins which takes place during the differentiative process (Dale et al., 1978). Another function ascribed to epidermal keratins is to impart mechanical integrity and structure to the epidermis as a tissue. Despite the widespread acceptance of this notion, however, direct in vivo demonstration has been difficult to obtain.

Elucidating keratin function has been aided by the recent demonstration that a truncated keratin missing 135 amino acid residues from the carboxy terminus of K14 (CΔ135-K14P) caused near complete disruption of keratin filament networks in basal epidermal keratinocytes of transgenic mice, and in addition, produced a phenotype that strongly resembled the Dowling-Meara subclass of the Epidermolysis Bullosa Simplex (EBS) family of human skin diseases (Vassar et al., 1991; Coulombe et al., 1991). All EBS patients are diagnosed by skin blistering induced by incidental trauma

1. *Abbreviations used in this paper*: EBS, epidermolysis bullosa simplex; IF, intermediate filament; PCR, polymerase chain reaction.

and arising from basal cell cytolysis (Fine et al., 1991). Dowling-Meara EBS is distinguished from other subtypes not only by an appreciable incidence of postnatal death, but also by the presence of large cytoplasmic clumps of tonofilaments (Anton-Lamprecht, 1983) that label with antibodies against the basal epidermal keratins (Vassar et al., 1991). Based on transgenic studies, it is tempting to speculate that it is the absence of a proper keratin filament network that contributes to loss of structural integrity, leading to cytolysis upon mild trauma. However, from these experiments alone, it was equally possible that accumulation of large aggregates of keratin leads to disorganization of cellular organelles, e.g., lysosomes, and subsequent cytolysis. This latter possibility has seemed especially likely in light of the rise in cytolysis that occurs developmentally at a time when K5 and K14 levels are also increasing (Vassar et al., 1991).

One way to distinguish between these two possible mechanisms for basal cell cytolysis would be to identify a keratin mutant that would still perpetrate cytolysis and elicit a reduction or perturbation in tonofilaments, but in the absence of appreciable tonofilament clumping. If it existed, such a mutant would be especially interesting, since these morphological features are shared by several milder EBS subtypes, including (*a*) Koebner, where less extensive, but recurrent, blistering occurs over many body regions, and (*b*) Weber-Cockayne, where blistering is nearly exclusive to palmar and plantar epidermis (for classifications, see Fine et al., 1991). A reduction or condensation of tonofilaments has been observed in milder EBS forms (Pearson, 1971; Haneke and Anton-Lampbrecht, 1982), although this is usually minimal and might be expected to be a logical consequence, rather than a cause of lysis. In fact, with the exception of one study postulating a delayed expression of keratins (Ito et al., 1991), reduction in filament numbers has not been considered a likely cause for skin blistering in Koebner and Weber-Cockayne EBS. Rather, it has been suspected that these diseases may be genetically unrelated to Dowling-Meara EBS and may arise, e.g., from defects in proteases or glycosylation (Savolainen et al., 1981; Sanchez et al., 1983; Fine and Griffith, 1985). Hence, despite the association between Dowling-Meara and mutations in the keratin K14 gene (Vassar et al., 1991; Coulombe et al., 1991), the possibility of generating other EBS phenotypes with alternative mutations in this gene did not seem likely.

Given the potential for providing insights into both the genetic relation among EBS subclasses and keratin function, we conducted additional transgenic mouse studies, this time targeting expression of three COOH-terminal and/or NH_2-terminal truncated K14 mutants, CΔ50K14P, NΔ117CΔ42-K14P, and CΔ135K14P, to stratified squamous epithelial tissues. Previously, we had shown that these three mutants perturb keratin filament network formation to varying degrees when transfected into cultured human keratinocytes (Albers and Fuchs, 1987, 1989). In addition, we demonstrated that these mutants all affect keratin filament elongation and stability in vitro, with CΔ50K14P having less severe effects than mutants similar to NΔ117CΔ42K14P, which had less severe effects than CΔ135K14P (Coulombe et al., 1990). We now show that in contrast to high-expressing CΔ135K14P transgenic mice, mice expressing elevated levels of NΔ117-CΔ42K14P and low levels of CΔ135K14P are viable, and exhibit biochemical and morphological features of the milder forms of EBS. Most importantly, our studies (*a*) demonstrate that phenotypes of both mild and severe subclasses of EBS can be generated from different mutations in a single (K14) gene, (*b*) document that filament network perturbation is an essential component of most if not all subclasses; (*c*) provide a molecular understanding of a fascinating but unexplained feature of EBS, namely blister recovery by cells prone to lysis; and (*d*) offer insights into the mechanism of cytolysis, which in turn has led to convincing evidence that a major function of keratin filaments is to impart cell shape–dependent mechanical integrity, without which basal cells lyse in response to mild physical trauma.

Materials and Methods

Plasmid Construction for the Transgenes

All plasmids contain inserts in pGEM3Z. Plasmid K14P (Vassar et al., 1989) contains sequences extending from ~2,300 bp 5′ upstream to the transcription initiation site of the human K14 gene, the coding sequence of human K14 cDNA minus sequences encoding the COOH-terminal 5 amino acid residues, sequences encoding the 15 amino acid neuropeptide substance P tag, and ~800 bp, including polyadenylation signal, of 3′ noncoding and downstream sequences from the human K14 gene. Plasmid CΔ50K14P is identical to pK14P, with the exception that sequences encoding 50 amino acid residues of K14 just before the P-tag were deleted. Plasmid NΔ117CΔ42K14P is identical to pK14P, except it is missing 200 nucleotides of 5′ upstream K14 gene sequence and it is missing sequences encoding the first 117 amino acid residues of K14, as well as sequences encoding 42 residues of K14 just before the P-tag. Plasmid CΔ135K14P (Vassar et al., 1991) contains sequences extending ~6,000 bp 5′ from the transcription initiation site of the human K14 gene, the first five exons and first four introns of the human K14 gene, and the remaining sequences of K14P up to those encoding 135 amino acid residues just before the tag.

Intermediate Filament Protein Extraction, Electrophoresis, and Immunoblot Analysis

IF proteins were isolated and protein concentrations were determined as described (Vassar et al., 1991). Proteins were resolved using SDS-PAGE, and either (*a*) stained with Coomassie blue or (*b*) electrophoretically transferred to nitrocellulose paper for immunoblot analysis (Vassar et al., 1991). Antisera used were (*a*) anti-P rabbit polyclonal (Wako Chemicals, Dallas, TX) and (*b*) anti-K14 rabbit polyclonal (Stoler et al., 1988). Primary antibody binding was detected with alkaline phosphatase–coupled goat anti-rabbit IgG (Bio-Rad Laboratories, Richmond, CA), according to the manufacturer's specifications.

Preparation and Identification of Transgenic Mice

Mutant keratin genes were isolated from plasmid expression vectors and microinjected into male pronuclei of single cell embryos from an outbred strain (CD-1) of mice (Charles River Laboratories, Inc., Wilmington, MA). Isolation of embryos and microinjection methods were as described (Vassar et al., 1989). Mouse ear or tail DNAs were isolated and assayed by PCR analysis for the presence of human K14P sequences. Two sets of primers were used: (*a*) a set specific for the 5′ upstream sequence of the human K14 gene, generating a diagnostic transgene band; and (*b*) a set specific for the K14 coding sequence, generating bands of different sizes for the endogenous mouse K14 and for each of the transgene keratin constructs.

Immunohistochemistry

Light Microscopy Level. Tissues were fixed, sectioned, and stained as described previously (Vassar et al., 1991). Primary antibodies used for staining were: (*a*) a 1:200 dilution of anti-K14 (Stoler et al., 1988); (*b*) a 1:100 dilution of a rat mAb NCI/34, against the COOH-terminus of neuropeptide substance P (Accurate Corp., Westbury, NY); (*c*) a 1:100 dilution of a

mouse monoclonal antibody specific for mouse K1 (courtesy of Stuart Yuspa, National Institutes of Health).

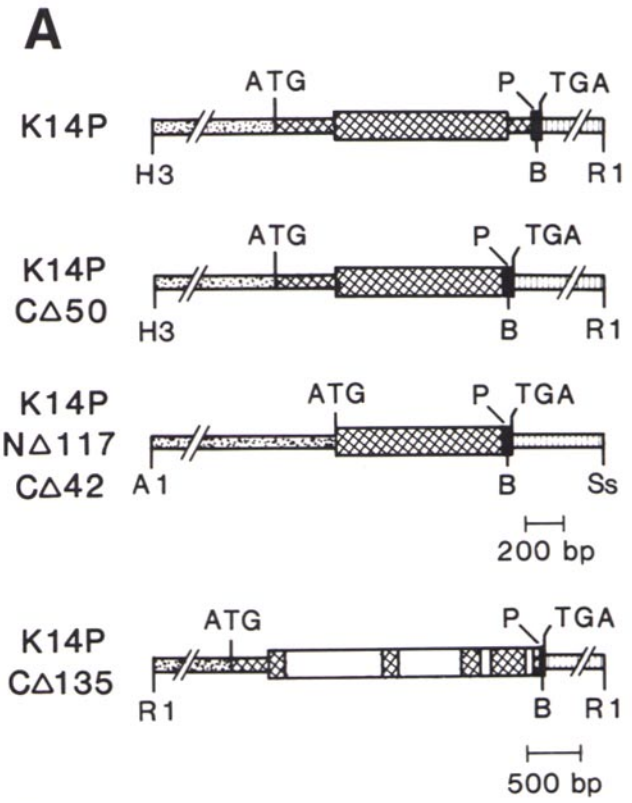
Electron Microscopy Level. Mouse or EBS human tissues were prepared for either routine ultrastructural studies (fixation in glutaraldehyde and osmium tetroxide, embedding in LX-112) or immunoelectron microscopy (fixation in paraformaldehyde, embedding in Lowicryl K4M) as described previously (Coulombe et al., 1989). Antibodies used for immunogold labelings were (*a*) rabbit polyclonal anti-P antiserum (Wako Chemicals, Dallas, TX), 1:200 dilution; and (*b*) rabbit polyclonal anti-K14 antiserum (Stoler et al., 1988), 1:300 dilution. 15-nm gold particle–conjugated secondary antibodies (Amersham Corporation, Arlington Hts., IL) were used, followed by washing, drying, and staining as described (Vassar et al., 1991). Prepared grids were examined using a Philips CM10 electron microscope. The specificity of cytochemical labeling was assessed through (*a*) repeating the above procedure using preimmune sera, rather than primary antikeratin antisera; and (*b*) repeating the above procedure, but omitting the primary antisera incubation step.

Results

Construction of Mutant Keratin Genes and Production of Transgenic Mice

The expression vector constructs used for our studies are illustrated in Fig. 1 *A*. A minimum of 2,100 bp 5′ upstream human K14 gene sequences were used to drive expression of truncated K14 proteins. Each transgene protein contained a COOH-terminal substance P tag in place of the corresponding K14 antigenic determinant, thus, permitting monospecific anti-P antibodies to track expression of the transgene product, and a polyclonal antiserum to the K14 COOH-terminus to track expression of endogenous mouse K14 (Vassar et al., 1989). The three mutant proteins lacked segments of the COOH-terminal and/or NH_2-terminal ends of K14. NΔ117CΔ42K14P lacked nearly the entire nonhelical carboxy- and amino-terminal ends of K14 and, in addition, ~2–10 residues at the amino end of the α-helical central rod domain (Albers and Fuchs, 1989; Coulombe et al., 1990). CΔ135K14P lacked the entire nonhelical carboxy tail and >30% of the carboxy end of the rod (Albers and Fuchs, 1987). CΔ50K14P lacked the entire nonhelical carboxy tail and ~8–10 residues of the rod (Albers and Fuchs, 1987). Using transient gene transfection studies, these mutants were previously shown to disrupt endogenous keratin filament networks to varying degrees, with CΔ135K14P > NΔ117-CΔ42- K14P > CΔ50K14P (Albers and Fuchs, 1987, 1989). In vitro assembly studies using purified bacterially expressed proteins showed that these mutants interfere predominantly with filament elongation and stability, again with the same order of severity (Coulombe et al., 1990).

Transgenic mice were produced using an outbred strain (CD-1) of mice as described previously (Vassar et al., 1989). 21 founder mice were transgenic, as judged by polymerase chain reaction (PCR) analysis of their skin DNAs. Six of these were examined previously in detail, and expressed CΔ135K14P at 5–80% endogenous K14 levels (Vassar et al., 1991). One new CΔ135K14P founder mouse was used for mating to generate transgenic offspring (CΔ135K14P-AF1). Six NΔ117CΔ42K14P founder mice were chosen for analysis: two were full transgenics and died within 15 h after birth; one (NΔ117CΔ42K14P-C) was a full transgenic and was bred for two generations (CF1 and CF2, respectively); and one was mosaic and was bred to produce full transgenic offspring (NΔ117CΔ42K14P-EF1). Finally, two CΔ50K14P



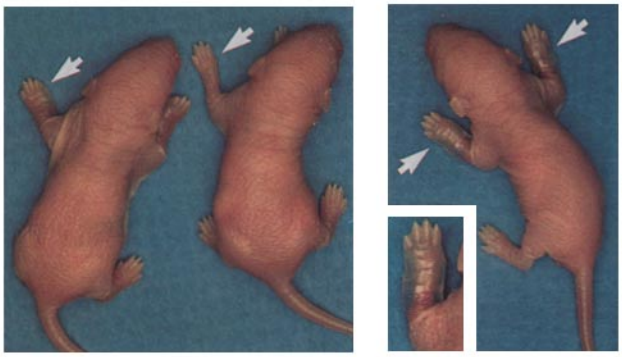


Figure 1. Genetic maps of expression plasmids and phenotype of NΔ117CΔ42K14P and low-expressing CΔ135K14P transgenic mice. (*A*). Plasmids are described in Materials and Methods. The components of these plasmids are: (*stippled box*) 5′ upstream sequence of the human K14 gene (Marchuk et al., 1984) extending from the ATG translation initiation codon to either a HindIII site at 2.3 kb, and AvaI site at 2.1 kb or an EcoRI site at ~6 kb 5′ to the TATA box; (*cross-stitched boxes*) the coding sequence of the human K14P or truncated cDNAs; (*open boxes*) the first four introns of the K14 gene; (*black box*) the sequence coding for the substance P tag; (*vertically striped box*) K14 3′ untranslated sequence extending from 21 bp 3′ from the TGA stop codon to the poly(A) signal (pA) and extending ~800 bp 3′ from pA. A1, AvaI; B, BamHI; RI, EcoRI; H3, HindIII; Ss, SspI. (*B*). (*left*) 2-d-old offspring from the F1 generation of mosaic mouse NΔ117CΔ42K14P-C. (*middle*) Nontransgenic littermate of transgenic mouse at left. (*right*, 2-d-old offspring of the F1 generation of transgenic mouse CΔ135K14P-A. Note the presence of epidermal blistering around the front paws of the two different transgenic animals. Arrows denote front paw blisters in transgenic animals or lack thereof in nontransgenic control. Inset shows blistered paw at higher magnification.

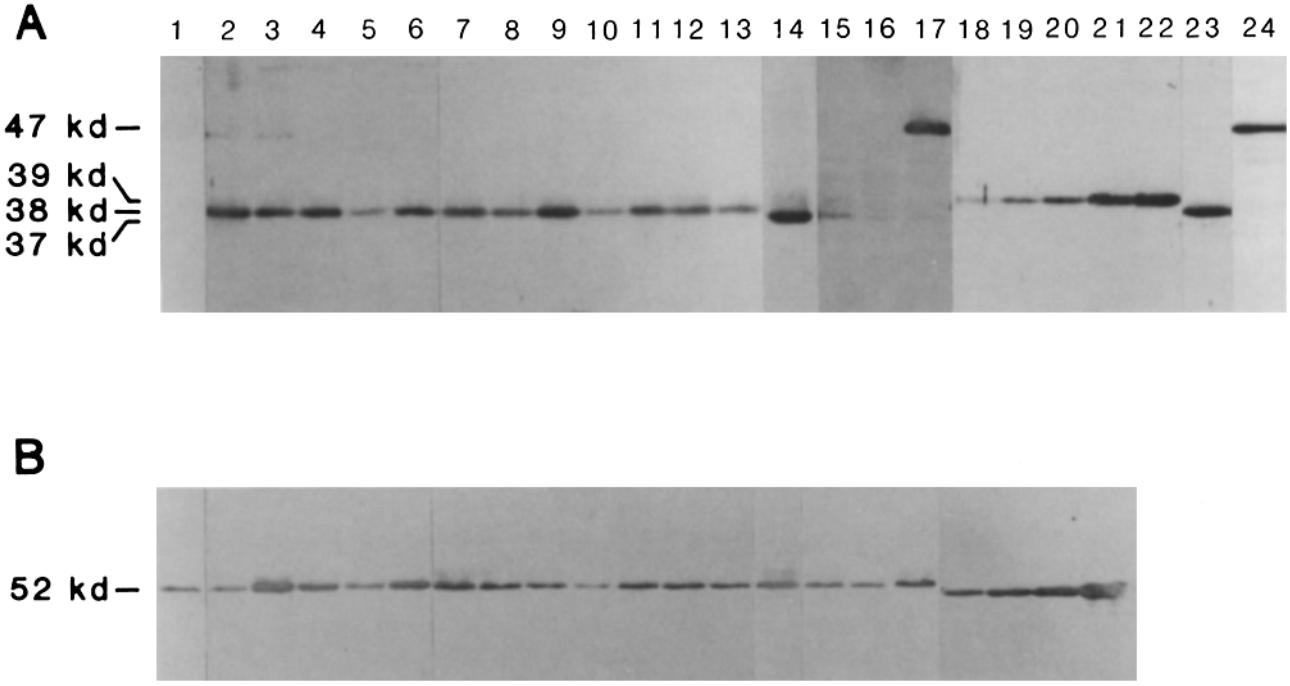


Figure 2. Anti-P and anti-K14 immunoblots of IF proteins from NΔ117CΔ42K14P, CΔ135K14P and CΔ50K14P transgenic mice and F1 and F2 offspring. If proteins were extracted and resolved by electrophoresis through triplicate SDS 11.5% polyacrylamide gels. Proteins from two gels (*A* and *B*) were subjected to immunoblot analysis using an anti-P (*A*) or anti-K14 (*B*) antiserum. The other gel (not shown) was stained with Coomassie blue. Samples in lanes *1–17* are same for both blots: lane *1*, control mouse, 7-d-old; lanes *2–6*, five founder NΔ117CΔ42K14P mice (*A–E*, respectively); lanes *7–9*, three F1 offspring of NΔ117CΔ42K14P founder mouse C (CF1a, CF1b, and CF1c, respectively); lanes *10–13*, four F2 offspring of NΔ117CΔ42K14P mouse C (CF2a, CF2b, CF2c and CF2d, respectively); lane *14*, high-expressing CΔ135K14P founder mouse 2B (for additional examples, see Vassar et al., 1991); lanes *15–16*, two F1 offspring of low-expressing CΔ135K14P founder mouse A (AF1a and AF1b, respectively); lane *17*, homozygous F2 offspring of CΔ50K14P mouse A (AF2a). Loadings were: 1 μg IF extract per lane for the K14 blot, and 15 μg IF extract per lane for the P blot, except for lane 14 of the P blot, where 5 μg was loaded. Remaining lanes contain standard proteins purified from overexpressing bacteria (Coulombe et al., 1990) (lanes *18–22* of *top gel*) NΔ107CΔ42K14P protein loaded at 10, 20, 60, 100, and 200 ng, respectively. (lane *23* of *top gel*) 60 ng CΔ135K14P protein. (lane *24* of *top gel*) 60 ng of CΔ50K14P protein. (lanes *18–21* of *bottom gel*) human K14 protein, loaded at 20, 60, 100, and 200 ng, respectively. Molecular masses of truncated proteins based on electrophoretic mobility were 39 kD (NΔ107CΔ42K14P), 38 kD (NΔ117CΔ42K14P), 37 kD (CΔ135K14P), and 47 kD (CΔ50K14P). Note: Mouse K14 runs as a 52-kD protein, whereas human K14 runs as a 50-kD protein.

founder mice were bred to homozygosity (CΔ50K14P-AF2 and -BF2).

Localized Palmar Blistering: A Hallmark of Milder Forms of EBS and a Characteristic of NΔ117CΔ42 Transgenic Mice

Within 1–2 d after birth, full transgenic offspring of NΔ117-CΔ42K14P founder mice exhibited palmar skin blistering on their front paws (Fig. 1 *B*, 2-d-old transgenic at left; compare with control littermate in center). This phenotype was also seen in F1 offspring of the CΔ135K14P mouse (Fig. 1 *B*, 2-d-old transgenic at right; *inset* illustrates paw blistering at higher magnification) but not in CΔ50K14P homozygous mice (not shown), which by sight were indistinguishable from control mice. The localized skin abnormalities were in marked contrast to previously studied CΔ135K14P founder mice, which exhibited gross blistering over whole body trunk skin at birth (Vassar et al., 1991). Moreover, whereas the previous CΔ135K14P mice died shortly after birth, many of these mice lived and hence could be analyzed during postnatal development.

Interestingly, blistering subsided after ~1–3 wk, coinciding with the development of a thick hair covering. Although the underside of the paws have fewer hair follicles than elsewhere, it seems plausible that hair growth might protect and/or stabilize the underlying epidermis. Consistent with this notion was the finding that incidences of recurrent paw blistering were rare in adults.

An additional clue as to why palmar skin might blister more than body trunk epidermis came from the development of ear, face, and neck wounds in adult mice. These wounds were sometimes recurrent, and they seemed to be initiated by scratching and/or rubbing, followed by infection. The pattern of blistering in both neonates and adult transgenic mice suggested that those skin regions subjected to frequent mechanical trauma were most sensitive to blistering. Collectively, the blistered paw phenotype, the ability of blisters to recover, and the ability to generate infection-prone wounds in response to mechanical trauma bore notable resemblance to milder subtypes of EBS, such as Weber-Cockayne and Koebner. Species-related differences in hairiness and in grooming habits, respectively, could account for (*a*) why pal-

mar and plantar blisters recur more frequently in EBS humans than in mice, and (*b*) why facial blisters were more prevalent in mice.

Severity of EBS Phenotype Correlates with Type and Level of Mutant K14

To measure levels of mutant K14P relative to endogenous K14, we isolated IF proteins from transgenic epidermises and prepared two immunoblots: one was probed with anti-P, specific for the transgene product (Fig. 2 *A*), and the other was probed with anti-K14, specific for the COOH-terminal peptide identical in both mouse and human K14 (Fig. 2 *B*). All transgenic samples contained diagnostic bands of the correct electrophoretic mobility and anti-P cross-reactivity (Fig. 2 *A*, lanes *2–17*; compare with aliquots of bacterially expressed and purified mutant proteins in lanes *18–24*). Using protein standards from bacterially expressed human K14, we estimated mouse K14 levels in each extract (Fig. 2 *B*, lanes *2–17*; compare with aliquots of human K14 protein in lanes *18–21*). After adjustments for molecular mass and loading differences, levels of mutant keratins relative to endogenous K14 were determined.

Data obtained from four independent immunoblot sets revealed that all F1 and F2 offspring of mouse C expressed NΔ117CΔ42K14P at 15–35% endogenous K14 levels (Fig. 2, lanes *7–13*). These mice all showed front paw blistering within 1–2 d after birth. In contrast, mosaic founder mice B and E (Fig. 2, lanes *3* and *6*, respectively) and two full transgenics (mouse C, Fig. 2, lane *4* and not shown) expressed NΔ117CΔ42K14P at 5–15% endogenous K14 levels. None of these animals displayed front paw blistering, suggesting that a critical level of mutant was necessary to generate the observed phenotype. Supporting this notion was the finding that the two new CΔ135K14P mice expressed only 1–2% of their K14 protein as mutant and exhibited front paw blistering (Fig. 2, lanes *15* and *16*), whereas our previously studied CΔ135K14P mice expressed 5–80% of their K14 protein as mutant, and exhibited total body trunk blistering (Vassar et al., 1991; for example, see Fig. 2, lane *14*, mutant 30% of wild type K14). Finally, two independent CΔ50K14P founder mice were bread to homozygosity, and at 15–35%, this mutant protein did not seem to be sufficient to generate a visible phenotype (Fig. 2, lane *17*). Collectively, our results revealed a correlation between severity of skin blistering and not only type, but also level, of K14 mutant. This was intriguing in light of previous studies demonstrating a parallel between levels of truncated keratins and the degree to which elongation of K5/K14 filaments was inhibited (Coulombe et al., 1990).

A Direct Correlation between Severity of Blistered Phenotype and the Degree to Which a Mutant Perturbs Keratin Filament Network Formation

To investigate the possible relation between severity of phenotype and extent of keratin filament network perturbations, we cultured keratinocytes from transgenic mice expressing varying levels and types of K14 mutants. Keratin networks were then examined by immunofluorescence (Fig. 3). Double immunofluorescence with anti-P and anti-K14 indicated colocalization of mutant and endogenous keratins. That colocalization implies copolymerization is likely, based on in vitro assembly studies with these mutant and wild-type keratins (Coulombe et al., 1990).

Keratinocytes cultured from NΔ117CΔ42K14P mice exhibited networks which were seemingly wild-type in the center, but showed ball-like structures at the periphery (Fig. 3, *A* and *C*, anti-P and *B*, anti-K5; compare with anti-K15 staining of control mouse keratinocyte in *D*). This phenotype was quite uniform within the population (Fig. 3 *C*) and, furthermore, it was seen for cultured keratinocytes from a variety of founder, F1 and F2 mice expressing in the range of 15% to >50% NΔ117CΔ42K14P. However, even within populations of full transgenic keratinocytes, there were occasional anti-P staining cells that displayed seemingly wild-type keratin networks (not shown), suggesting that a fine balance may exist between the normal and ball-like phenotypes in the mutant cells. Alternatively, even though the keratinocytes had the same genetic constitution, there may have been epigenetic events responsible for these variations within the population.

Cells expressing 1–2% CΔ135K14P exhibited a different keratin network, displaying fewer filaments and more cable-like structures (Fig. 3 *E*, anti-P). Thus, despite their ability to generate a phenotype similar to NΔ117CΔ42K14P mice in vivo, these keratinocytes exhibited distinct differences in vitro. As expected, keratinocytes cultured from mice expressing high levels of CΔ135K14P exhibited a network that seemed somewhat similar, but more grossly perturbed than keratinocytes from low-expressing CΔ135K14P mice (Fig. 3, compare *F*, anti-P staining of high-expressing line with *E*, the low-expressing line). Epidermal cells from mice expressing moderate levels of CΔ50K14P exhibited the mildest perturbations, which were similar in appearance to NΔ117-CΔ42K14P networks, but with smaller ball-like structures at the periphery (Fig. 3 *G*, anti-P). Thus, all mutant keratin networks appeared aberrant, and while differences were sometimes subtle, the degree of abnormality correlated with the degree to which these mutants interfered with keratin filament assembly in vitro (Coulombe et al., 1990). Most importantly, the extent of perturbation paralleled the level of skin blistering in vivo. Curiously, however, we found no evidence for lysis in NΔ117CΔ42K14P and low-expressing CΔ135K14P cultures. This was distinct from high-expressing CΔ135K14P cultures (Vassar et al., 1991) and from in vivo epidermis of mice expressing all levels of either of these two mutants. The possible basis for this will be discussed later.

Blistered Epidermis Recovers without Apparent Scarring: A Novel Feature Shared By EBS and Mutant Keratin Transgenic Mice

Previously, we discovered that high-expressing CΔ135K14P mice blister as a consequence of basal cell cytolysis, a classical feature in EBS patients (Vassar et al., 1991). Histopathological examination of paw blisters from NΔ117CΔ42K14P mice (shown) and low-expressing CΔ135K14P mice (not shown) revealed a clean separation of epidermis and dermis (Fig. 4, compare *A*, front paw with *B*, unaffected back paw). This blistering was due to basal cell cytolysis, as evident in semi-thin sections (0.75 μm) of epoxy-embedded tissue (Fig. 4 *C*). In contrast to most high-expressing CΔ135K14P mice

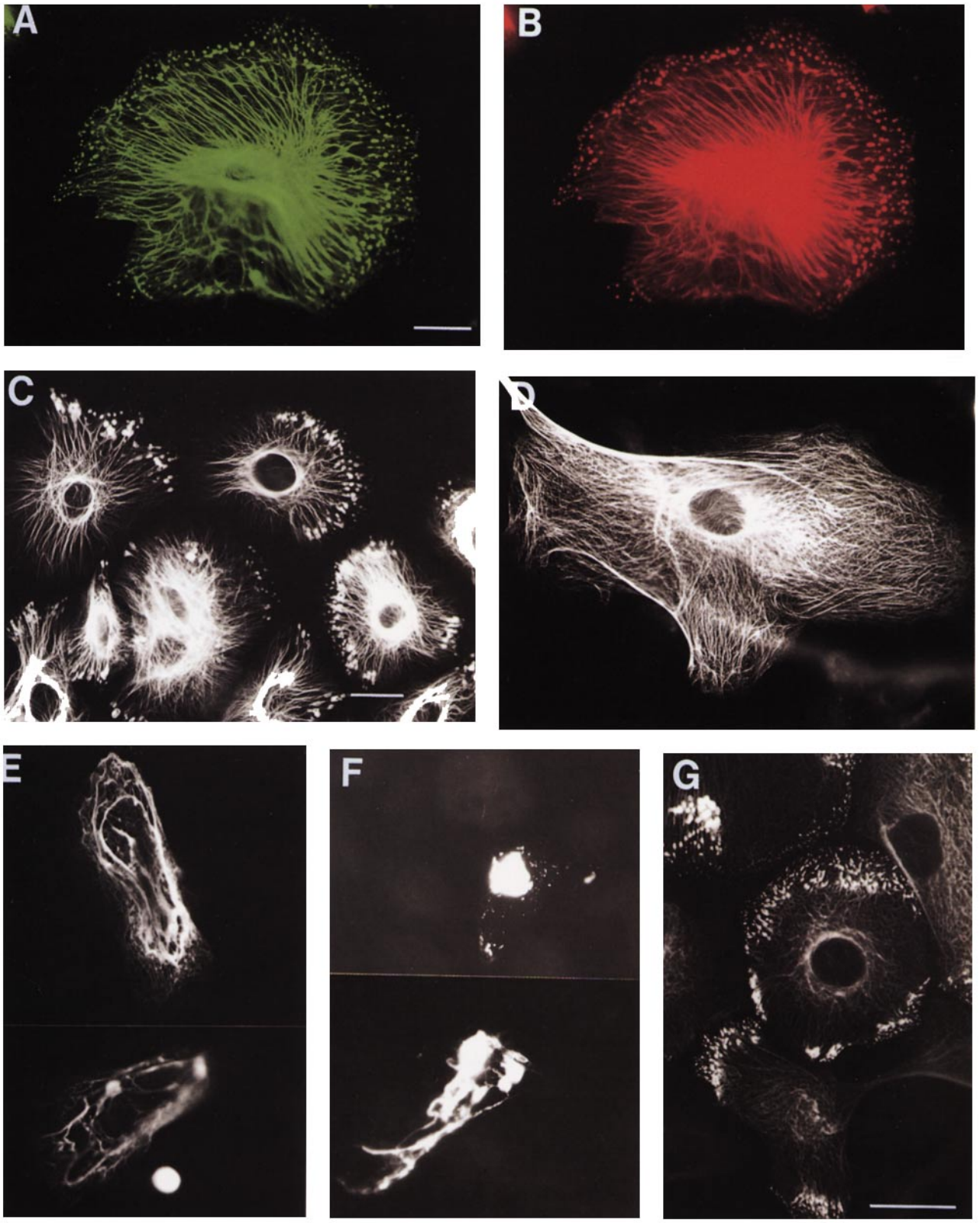


Figure 3. Cultured transgenic keratinocytes have variations in the extent of perturbation of their keratin filament networks. Epidermis from transgenic and control newborns were trypsinized, and cells were plated on mitomycin C-treated mouse 3T3 fibroblast feeder cells. Cells were fed on low calcium-containing medium as described by Hennings et al. (1980). NΔ117CΔ42K14P-expressing epidermal cells from the full transgenic founder mouse A (*A–C*), control mouse (*D*), low-expressing CΔ135K14P mouse AF1a (*E*), high-expressing CΔ135K14P mouse 2B (*F*), and CΔ50K14P mouse AF2a (*G*) were fixed and costained with rabbit anti-P (*A*, *C*, *E*, *F*, and *G*) and guinea pig anti-human K5 (*B* and *D*). Bound antibodies were visualized with fluorescein-conjugated anti-rabbit IgG antiserum and Texas red-conjugated anti-guinea pig IgG antiserum, respectively. Bar in *A* applies for *A* and *B*; bar in *C* applies for *C–F*. Bars: 50 μm.

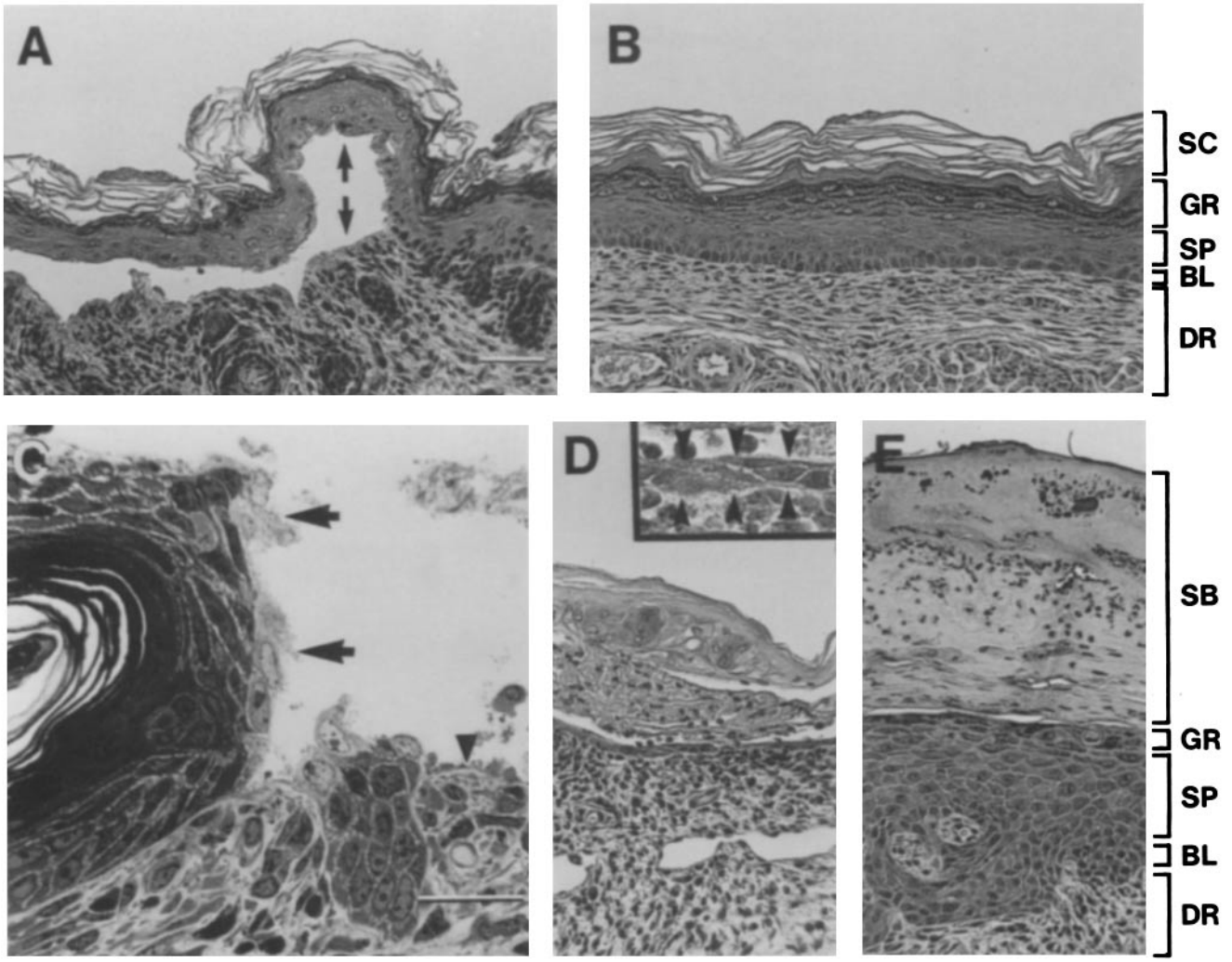


Figure 4. Hematoxylin-eosin and toluidine blue staining of sections of skin from NΔ117CΔ42K14P-expressing transgenic mice during and after blistering of paws. Skin from F1 and F2 offspring of NΔ117CΔ4214P-expressing transgenic mice and one nontransgenic control were prepared for light microscopy (see Materials and Methods). Sections were subjected to hematoxylin and eosin (*H/E*) or toluidine blue (*TB*) staining. (*A*) Section from front paw blister from mouse CF1a, H/E (2-d-old mouse); (*B*) section of unaffected back paw from same mouse, H/E; (*C*) semi-thin (0.75 μm) section of epoxy resin–embedded tissue of same animal as in *A* and *B*, TB; (*D*) section of front paw from mouse CF1b, during early stages of recovery (~2-d-old mouse), H/E. Note one to two layers of flattened basal-like cells, evident at higher magnification as illustrated in inset (between *arrowheads*) (*E*) section of front paw from mouse CF2a, during later stages of recovery (~7-d-old mouse), H/E. Bar in *A* represents 100 μm and applies for *A*, *B*, *D*, and *E*. Bar in *C* represents 50 μm and applies for *C* and inset to *D*. *SC*, stratum corneum; *GR*, granular layer; *SP*, spinous layers; *BL*, basal layer; *DR*, dermis; *SB*, scab. Arrowheads in *C* denote remnants of lysed basal cells still attached to the basement membrane; arrows denote those remnants attached to epidermis. Note typical presence of leukocytes and erythrocytes in scabs over paw skin in *D* and *E*.

(Vassar et al., 1991), NΔ117CΔ42K14P and low-expressing CΔ135K14P mice exhibited few abnormalities in the organization of suprabasal cell layers overlying the blister.

The generation of viable F1 and F2 offspring of NΔ117-CΔ42K14P mice enabled us to examine for the first time another hallmark of EBS, namely the unusual property of this skin to heal, and to do so without scarring (for review, see Fine et al., 1991). In the early stages of blister recovery, a single layer of flattened basal cells appeared beneath the scabs over the paw skin (Fig. 4 *D*, *inset* shows higher magnification of flattened basal cells). Examination of semi-thin sections of epidermis shortly after wound closure revealed numerous mitoses in the regenerating basal layer, indicative of a highly proliferative epidermis. Remarkably, as recovery continued, a new and seemingly healthy epidermis formed, complete with basal-like, spinous-like, and granular cells (Fig. 4 *E*). Notably, we did not detect cytolysis within the sections examined.

The Recovery Process of Blistered Epidermis May Involve Increased Protection against Trauma, Less Differentiated Basal Cells, and Changes in Cell Shape

It seemed surprising that cytolysing basal cells could regenerate an epidermis. However, the overlying scab could have lent some protection to the underlying, newly dividing basal cells. In addition, we noticed that animals with paw blisters

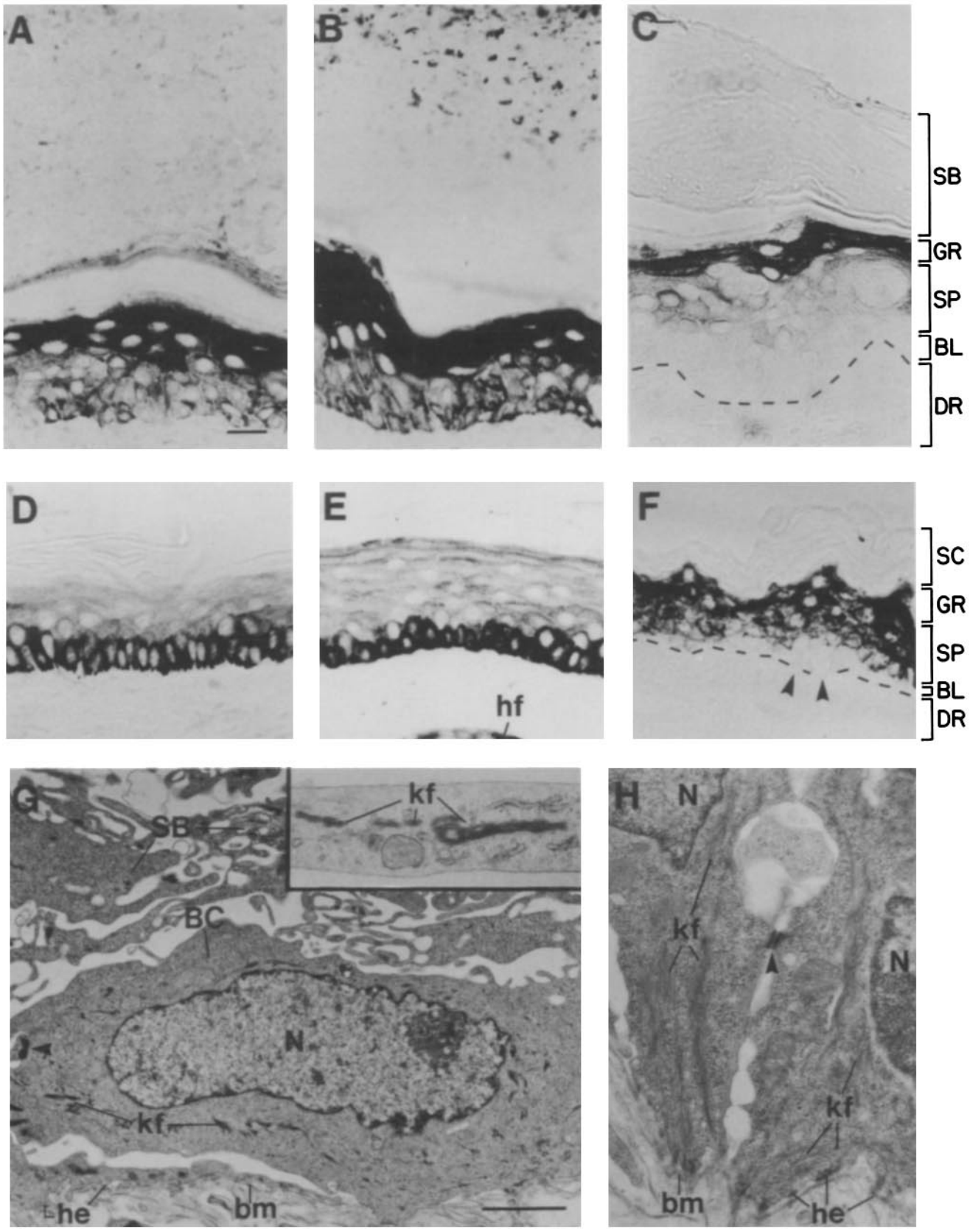


Figure 5. Abnormal keratin expression in recovering blister regions of transgenic mouse epidermis. Recovering and fresh blisters from skin of F1 or F2 offspring of NΔ117CΔ42K14P mouse C were taken at 7 d (recovering blister, *A–C* and *G*) or 2 d (fresh blister, *F*; see also Vassar et al., 1991). Sections of skin were also taken from a wild-type K14P-expressing transgenic mouse (*D* and *E*; see also Vassar et al., 1989), and from a nontransgenic control mouse taken at 7 *d* (*H*). Cultured NΔ117CΔ42K14P-expressing keratinocytes (*inset* to *G*) were from full transgenic founder mouse A. (*A–F*), Samples were fixed, paraffin-embedded, and sectioned as in legend to Fig. 4.

gingerly avoided physical trauma to these regions. Thus, overall there was probably less mechanical irritation to the recovering tissue, perhaps minimizing basal cell cytolysis.

To investigate whether there might also be changes in the biochemistry of recovering epidermis, we stained sections of recovering and blistered palmar skin with antibodies against (*a*) mutant transgene protein (P-tag), (*b*) endogenous K14, and (*c*) suprabasal keratin K1 (Fig. 5). Interestingly, the patterns of staining were markedly different for recovering epidermis (Fig. 5, *A–C*) and control or blistered epidermis (Fig. 5, *D–F*). As expected from our previous mutant mice studies (Vassar et al., 1991), staining patterns of blistered paw epidermis were quite similar to those of control skin from mice expressing "wild-type" K14P. Thus anti-P and anti-K14 staining was strong in basal cells (Fig. 5, *D* and *E*, respectively; K14P mouse) and anti-K1 staining was exclusive to the suprabasal layers (Fig. 5 *F*; blistered paw epidermis of NΔ117CΔ42K14P mouse). In contrast, recovering skin exhibited weak anti-P and anti-K14 staining in the basal layer, and strong staining in the mid to upper spinous layers (Fig. 5 *A* and *B*, respectively). Moreover, anti-K1 staining was only present in the upper suprabasal layers (Fig. 5 *C*).

The immunohistochemical studies suggested that the program of keratin expression may be delayed in cells migrating into blister sites, a notion that was supported by ultrastructural studies (Fig. 5 *G* and *H*). Indeed, basal cells in recovery zones (Fig. 5 *G*) showed substantially fewer tonofilaments than basal cells from nontransgenic skin (Fig. 5 *H*). Whether these cells represent an intermediate between stem and basal cells, whether they are outer root sheath cells that have not yet differentiated into basal epidermal cells, or whether they represent basal cells that have undergone dedifferentiation upon wounding awaits further investigation.

Recovering basal cells not only escaped lysis, but in the early phases, they were also more flattened and less columnar than normal (Fig. 5, compare size and dimensions of flattened cell in *G* with the part of the columnar ones at twice the magnification in *H*). Intriguingly, these features were also characteristic of our cultured NΔ117CΔ42K14P keratinocytes (Fig. 5, see *inset* to *G*; compare reduced height of this basal cell with that of recovery and control cells). Collectively, these data suggested that cell shape and perhaps intracellular concentrations of keratins play a role in susceptibility of a basal cell to rupture. In addition, suprabasal cells in recovery zones resembled basal cells in normal regions, and only in the upper layers contained the denser tonofilament bundles typical of spinous cells (data not shown). Despite significant levels of mutant and basal keratins in the apparent absence of K1/K10, however, these suprabasal cells did not show appreciable signs of cytolysis, thereby confirming that cytolysis must be dependent upon factors in addition to mutant keratin expression.

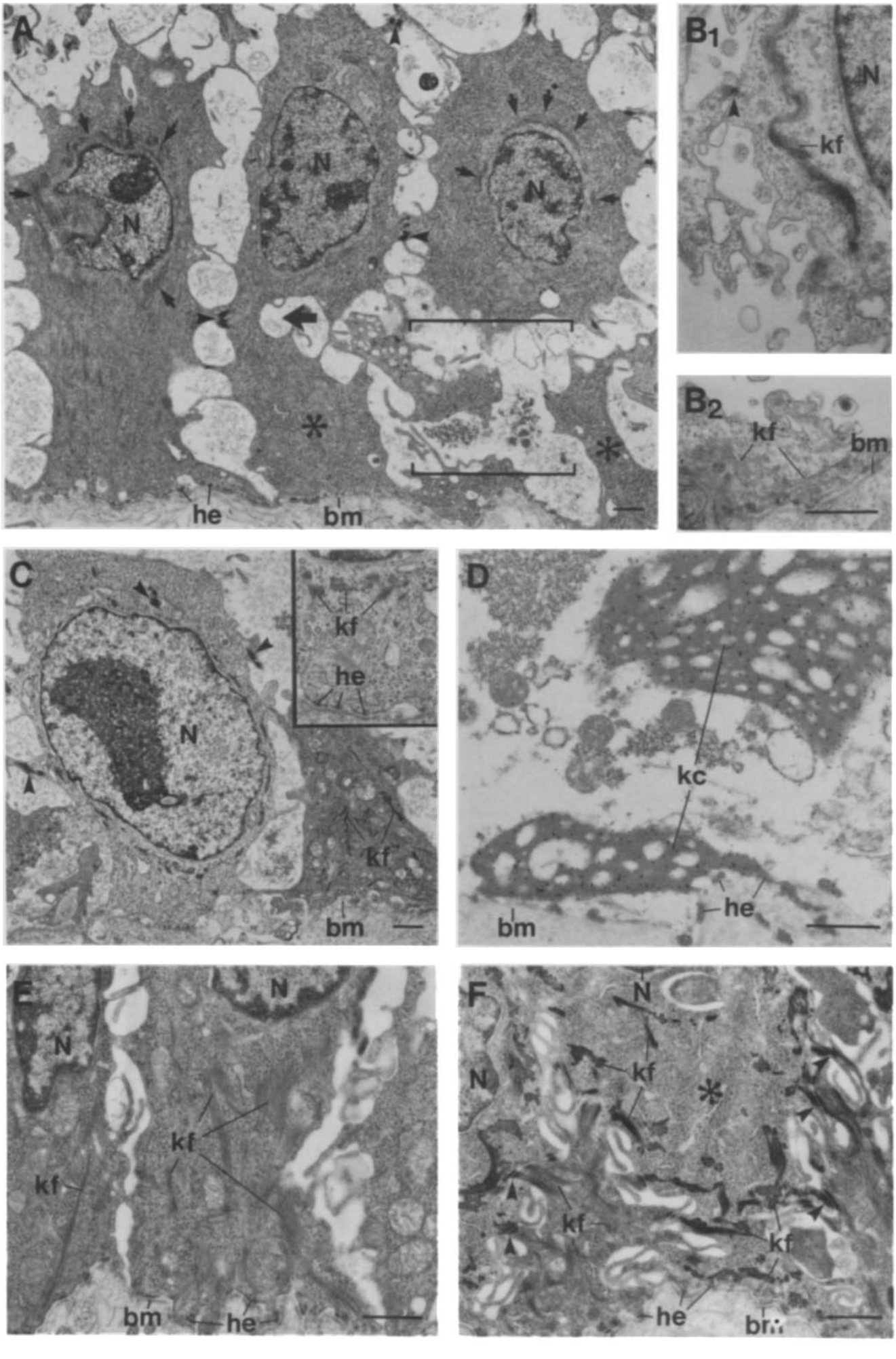
Basal Cell Cytolysis Occurs in a Defined Subnuclear Zone and Does Not Rely upon Production of Large Insoluble Aggregates of Keratin

The pathobiology of NΔ117CΔ42K14P and low-expressing CΔ135K14P transgenic mice resembled that of the Koebner and Weber-Cockayne subtypes of EBS (for review, see Fine et al., 1991). To explore further the relation between these EBS subtypes and NΔ117CΔ42K14P mice, we examined in detail the ultrastructure of epidermis from full transgenic mice (F1 and F2 offspring of mouse C). Typical of all subtypes of EBS, the most notable morphological aberrancies in low-expressing CΔ135K14P or NΔ117CΔ42K14P-expressing skin resided in the basal epidermal layer. Ultrastructural analysis revealed that cell rupture occurred in a narrowly defined region located midway between the nucleus and the hemidesmosomes (Fig. 6 *A*; note two cells at right). Subnuclear lysis has also been observed in Weber-Cockayne EBS skin (Haneke and Anton-Lamprecht, 1982). Thus, this region of the basal cell seemed to be particularly vulnerable both to our mutant-induced changes and to changes induced by the genetic defect(s) associated with EBS. These correlations were both striking and important, and were not evident in our earlier studies, where massive cytolytic destruction distorted tissue architecture (Vassar et al., 1991).

Basal cells in NΔ117CΔ42K14P mouse skin sections exhibited little or no evidence of tonofilament clumping. Moreover, in blistered regions tonofilaments were still prevalent, albeit reduced (Fig. 6 *B1*, top of ruptured basal cell; and *B2*, bottom of lysed cell). In addition, the organization of filaments was markedly perturbed, particularly in the ruptured zone and the regions near hemidesmosomal contacts (Fig. 6, *A*, *B2*, and *C*). While thin sectioning did not permit quantitation, tonofilament bundles seemed atypically short and did not extend to the base of the cell (compare example in Fig. 6 *C*, with control skin in Fig. 5 *H*). This finding was consistent with the knowledge that these mutants interfere with keratin filament elongation and stability in vitro (Coulombe et al., 1990). Overall, this ultrastructure was in contrast to that of lysing basal cells in Dowling Meara EBS and high-expressing CΔ135K14P transgenic mice, both of which showed very few tonofilaments and large clumps of keratin protein (Fig. 6, *D*, anti-P labeling; see also Vassar et al., 1991).

From an analysis of only blistered skin, it was difficult to determine the extent to which cell rupture might have contributed to cytoskeletal alterations. Therefore, we examined

These sections were subjected to immunostaining with anti-P (*A* and *D*), anti-K14 (*B* and *E*), or antimouse K1 (*C* and *F*). Note that anti-P and anti-K14 staining were reduced in the basal layer of the recovering region, but extended into the suprabasal layers. Note also that anti-K1 staining is delayed in the recovery zone. Final note: the patterns of antibody staining in freshly blistered regions were similar to those of unaffected regions and nontransgenic skin, with the exception that anti-P and anti-K14 staining extended, but was weaker in a few suprabasal layers as reported previously (Vassar et al., 1991). Bar in *A* represents 50 μm and applies to *A–F*. (*G* and *H*) Samples were processed for EM as described in Materials and Methods. Cross-sections of basal epidermal cells are shown. Note that basal cells in recovering zones (e.g., *G*) are healthy, but have fewer, thinner, and more randomly oriented tonofilament bundles than nontransgenic basal cells (*H*). Note that height of cultured basal cell (*inset* to *G*) < recovering basal cell (*G*) < nontransgenic columnar basal cell (*H*). *kf*, keratin filaments; *BC*, basal cell; *SB*, suprabasal cell; *bm*, basement membrane; *he*, hemidesmosome; *N*, nucleus; *hf*, hair follicle. Arrowheads denote desmosomes. Bar equals 2 μm for *G* and 1 μm for inset to *G* and for *H*.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back skin, which is protected by hair and subjected to less trauma than paw skin. In these regions, gross blistering was not present and cell lysis was minimal (Fig. 6, *E* and *F*). An interesting finding consistent with our cultured cell analysis was that even though 100% of the cells were transgene-expressing, many cells in nonblistered areas displayed cytoskeletal networks that were similar to control cells (Fig. 6 *E*). However, many cells exhibited atypical keratin filament networks, and similar to blistered regions, there was considerable heterogeneity in cytoarchitecture. As in obviously ruptured cells, many but not all, abnormalities were in the subnuclear zone (see example in Fig. 6 *F*). This included constrictions near the cell base, areas nearly devoid of tonofilaments (denoted by *asterisk* in Fig. 6 *F*), and short filament bundles oriented randomly rather than parallel to the nucleus–cell base axis. Although some heterogeneity in cytoskeletal architectures was evident in control skin sections, the degree was significantly greater in transgenic sections. We do not know whether these phenotypes represent multiple stages of a single pathway of cytoskeletal collapse. However, it is interesting that many of these ultrastructures, ranging from normal to markedly perturbed, were similar to those previously reported for EBS Weber-Cockayne and Koebner (Fig. 7 *A*, see also Pearson, 1971; Haneke and Anton-Lampbrecht, 1982).

We were somewhat surprised that we found little evidence in vivo for the ball-like structures at the periphery of cultured keratinocytes in vitro. In this regard, it was interesting that the Koebner EBS skin shown in Fig. 7 was also seemingly devoid of these structures and yet when cultured, aberrancies at the cell periphery appeared (Fig. 7 *B*). Ball-like structures at the periphery of cultured EBS keratinocytes are discernable at the EM level (Kitajima et al., 1989), and thus it seems likely that these structures are generated either when epidermal cells flatten and spread in culture or when they are fixed for immunofluorescence. Since these changes do not take place when control skin cells are cultured, they may be an indication of an as yet unidentified cytoskeletal interaction, weakened as a consequence of the keratin defects.

Overall, our keratin mutants generated quite subtle ultrastructural changes in vivo, and most of these seemed to be at the level of tonofilament organization within the cell. The findings have three implications: (*a*) basal cell cytolysis elicited by keratin mutations is not dependent upon gross tonofilament clumping; (*b*) even subtle perturbations in keratin networks can cause basal cell cytolysis upon incidental trauma; and (*c*) the similar phenotype in Weber-Cockayne and Koebner EBS could arise from keratin gene defects.

Discussion

Function of Intermediate Filaments: General and Epidermal-specific

Given the ubiquity and oftentimes abundance of IF proteins, it seems extraordinary that their function has remained elusive since their discovery in the early 1960s. Their diverse patterns of expression have revealed some clues for possible specialized functions, and although much of the evidence to support these roles is still speculative, the natures of these functions are becoming increasingly more apparent (Newport et al., 1990; Glass and Gerace, 1990; Monteiro et al., 1990; Weinstein et al., 1991). While IF heterogeneity is suggestive of multifarious functions, the common structure of these filaments also implies a shared function which cannot be a housekeeping role, given that several cultured cell lines lack cytoplasmic IFs altogether (Venetianer et al., 1982; Hedberg and Chen, 1986). One possible common function for IFs might be to provide mechanical integrity to the nucleus (lamins) or cytoplasm (other IFs). Interestingly, a *Xenopus* oocyte extract depleted of its single nuclear lamin could encapsulate chromatin and assemble nuclear pores, but the resulting nuclei were fragile (Newport et al., 1990). In contrast, however, disruption of the vimentin network in microinjected frog embryos was without apparent consequence to early development (Christian et al., 1990). If vimentin IFs have a function in early development, it may be that the functional redundancy imparted by the coexistence of simple epithelial keratin filaments masked a vital role for these filaments in vivo.

Our initial studies on epidermal keratin mutants in transgenic animals could not address the question of function, because not only did the mutant CΔ135K14P disrupt the keratin filament network in basal cells, but in addition it generated large clumps of keratin protein. In contrast, mutant NΔ117CΔ42K14P had a much less severe impact on keratin network formation, often leading to tonofilament disorganization, but with no large aggregates. Since basal cell lysis was still prevalent in both types of mutant mice, we can no longer attribute mutant keratin–mediated cytolysis to the

Figure 6. Ultrastructure and immunoelectron microscopy of basal cells from NΔ117CΔ42K14P and high-expressing CΔ135K14P full transgenic mice. Blistered paw and nonblistered back skins were from three 0–7-d-old full transgenic mice. Skins were fixed and sectioned for routine and immunogold EM (Materials and Methods). (*A–C*) Affected front paw skins of NΔ117CΔ42K14P mice. (*A*) Low magnification survey showing three columnar-shaped basal cells. Cell at right appears lysed, and the plane of rupture lies between the hemidesmosomes and the nucleus (see *brackets*). Center cell appears to be in process of rupturing (see *thick arrow*), while the left cell shows no signs of lysis. Typically, the basal-most portion of cytoplasm is devoid of tonofilament bundles (*asterisk*), which oftentimes appear coiled around the nucleus (*short arrows*). (*B1–B2*) Top and bottom portions, respectively, of blistered cells. Again, plane of lysis is subnuclear. Note larger tonofilament bundles in upper portion, shorter and thinner tonofilament bundles in lower segment, but no tonofilament clumping anywhere. (*C*) Degenerating, but seemingly unlysed, basal cell. Inset denotes short filament bundles; note that they do not extend to the hemidesmosomes. (*D*) Blistered skin of high-expressing CΔ135K14P mouse processed for immunoelectron microscopy (Materials and Methods). Labeling was achieved using anti-P antibodies. Note presence of extensive keratin clumps (*kc*), densely labeled with gold particles. (*E–F*) Unaffected backskin of NΔ117CΔ42K14P mice. (*E*) Seemingly unaffected basal cell with near normal tonofilament bundles. Such apparently normal cells were seen in both NΔ117CΔ42K14P and low-expressing CΔ135K14P mice, even though they were full transgenics. (*F*) Basal cell with completely disorganized keratin filament network. Asterisk denotes large region of cytoplasm devoid of filaments. Additional abbreviations are same as in Fig. 5 legend. Bars: 1 μm.

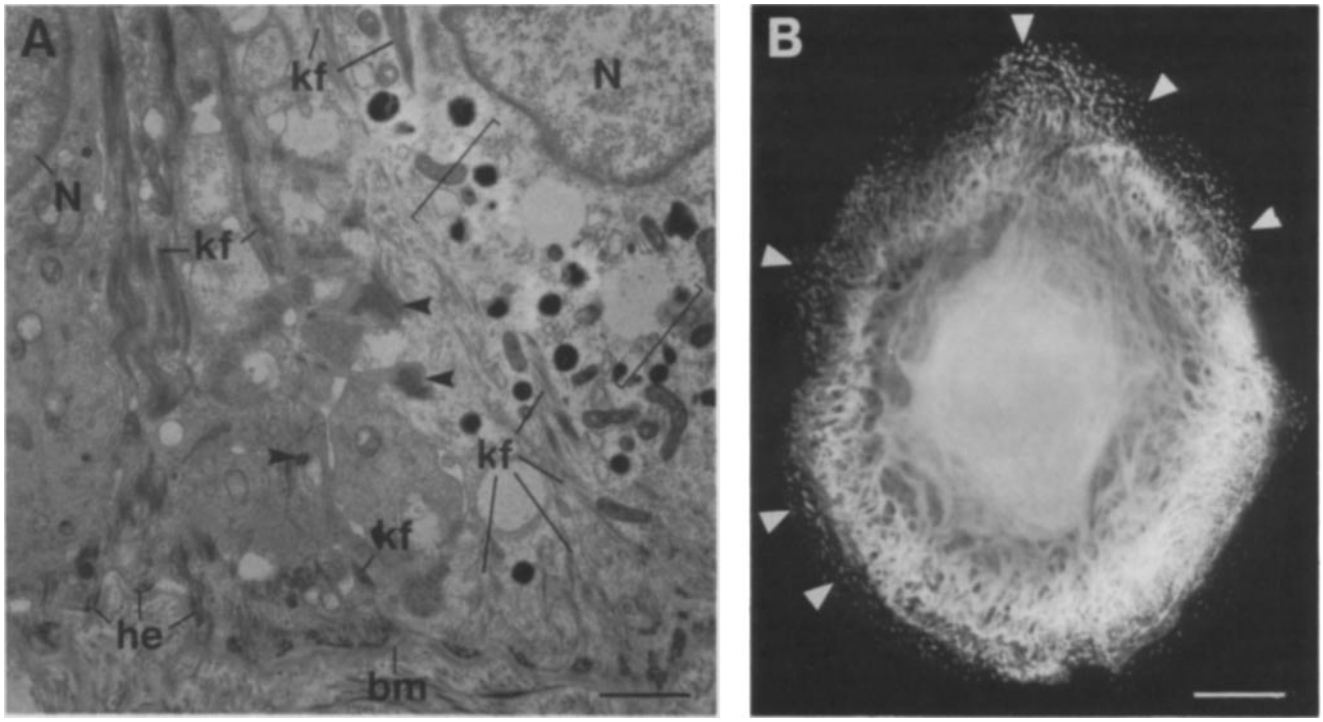

Figure 7. EM and immunofluorescence of skin sections and cultured epidermal cells, respectively, from a patient with epidermolysis bullosa simplex (*EBS*) Koebner. (*A*) Skin from a nonblistered region of a patient with EBS Koebner was fixed and processed for EM as described in Materials and Methods. (*A*) Region from basal layer. Note presence of seemingly unlysed cell with normal looking tonofilament network (*left*) adjacent to degenerating cell with fewer, shorter and thinner tonofilament bundles (*right*). Brackets denote subnuclear zone of cell fragility. Note also that electron-dense spherical bodies are melanin granules, not seen in basal cells from albino transgenic animals. Abbreviations are as in legend to Fig. 5. (*B*) Epidermal cells from skin of same patient were cultured and stained with anti-K14 antiserum. Arrowheads denote abnormal keratin staining at cell periphery. Bars: (*A*) 1 μm; (*B*) 50 μm.

formation of large insoluble aggregates. Moreover, given the marked correlation between the occurrence of both tonofilament disorganization and cell rupture in a defined subnuclear zone of basal cells, it seems most likely that it is the perturbation of a proper keratin filament network that leads to cytolysis.

An important question left unanswered is how cytolysis occurs. Previous studies on EBS patients have convincingly demonstrated that mechanical trauma is an important component, and our transgenic mouse studies provide additional evidence to support this. However, several lines of evidence have led us to suspect that the cytoarchitecture of the cell is also intricately involved. In this regard, it is relevant that in both cases where NΔ117CΔ42K14P basal cells did not lyse, i.e., in cell culture and during blister recovery, the cells were flattened rather than columnar in shape. Having a greater surface to volume ratio, flattened cells might be able to better resist rupture than columnar cells, a feature that might also explain why cultured cell lines lacking cytoplasmic intermediate filaments altogether are viable. Coupled with the fact that both zone of rupture and tonofilament disorganization in NΔ117CΔ42K14P mouse and EBS cells seemed to be localized, these observations point to the hypothesis that it was a specific cytoskeletal architecture of columnar, but not flattened basal cells, which was compromised by our keratin mutations and by the genetic defect(s) in EBS patients.

As documented by the number of seemingly normal or only mildly affected transgenic keratinocytes in vivo, the perturbation to cytoarchitecture need not be a gross one. Indeed, there were still numerous keratin filaments present, even in the lysing cells of transgenic skins. Based on our previous in vitro assembly studies, these mutants all perturb filament elongation and IF stability (Coulombe et al., 1990), and while it is difficult to assess from an EM study of thin sections, transgenic filaments seemed to be shorter than normal. It seems plausible that a moderate reduction in keratin filament length and/or efficiency of polymerization might be sufficient to cause localized weakening of the network, but further studies will be necessary to elucidate why this zone is so vulnerable, and specifically how this weakening occurs.

Appraisal of the structural integrity of a cell might require not only maintenance of a critical cell shape, but also an environment that can be perturbed, i.e., one that in vivo, might only be characteristic of superficial tissues, including epidermis and hairs. In this regard, it is interesting that when keratin filament organization is altered in transgenic hairs, it leads to brittleness, a condition which might also be related to a weakened cellular architecture (Powell and Rogers, 1990). A final point relevant to the notion that cytoplasmic IFs contribute to structural integrity is a recent evaluation of the viscoelastic properties of vimentin, demonstrating that these IFs are flexible at low strain, but they harden at high strain and resist breakage under stresses where actin networks would be ruptured (Janmey et al., 1991). Thus, while gene ablation experiments have not yet been conducted for cytoplasmic IFs, and while functional redundancy may complicate the interpretation of such studies, it is becoming apparent from other avenues that like lamins, cytoplasmic IFs

play a role in maintaining the architectural framework of a cell in the context of its tissue. The extent to which this function is shared by all cell types remains to be shown, but from our studies on epidermal keratins, it is likely to depend upon the cell shape, the environment and the functional redundancy imparted by (*a*) multiple IF networks and (*b*) other cytoskeletal components.

Are EBS Subtypes Genetically Related and Do They Involve Defects in K5 and K14 Genes?

The extent to which different subtypes of EBS are genetically related is unknown. Etiological postulates include alterations in proteases and glycosylation (EBS-Koebner; Savolainen et al., 1981; Sanchez 1983; Manabe et al., 1984), aberrancies in the regulation of keratin expression (EBS-Koebner; Ito et al., 1991), postsynthetic modification of keratins (EBS-Dowling-Meara; Tidman et al., 1988), and structural defects in keratin (Dowling-Meara and severe forms of Koebner; Anton-Lamprecht, 1983; Kitajima et al., 1989; Vassar et al., 1991; Coulombe et al., 1991). Prior to the present study, keratin gene defects have only been associated with the Dowling Meara subtype of EBS (Vassar et al., 1991; Coulombe et al., 1991). Thus, the mutant CΔ135K14P gave rise to particularly severe Dowling-Meara EBS in transgenic mice (Vassar et al., 1991), and point mutations in a critical region of the K14 protein sequence were found in two spontaneous cases of human Dowling Meara EBS (Coulombe et al., 1991). Our present results convincingly demonstrate that various lesions in a single, K14, keratin gene can mimic most morphological features of the milder subtypes of EBS, including Weber-Cockayne and Koebner. In addition, our findings provide plausible explanations for many of the previously poorly understood features of the EBS phenotype. Thus, for example, the realization that keratin expression is delayed during blister recovery in our transgenic mice provides a possible explanation for why Ito et al. (1991) proposed that EBS Koebner might be a genetic defect in delayed keratin expression. Moreover, the surprising finding that keratin gene defects can sometimes elicit very subtle changes in a basal keratin network and still give rise to cytolysis, illustrates why most researchers may have overlooked keratin gene defects as a likely basis for the milder forms of EBS. However, despite our ability to clearly implicate aberrancies in keratin filament cytoarchitecture as a basis for EBS, our data do not rule out the possibility that EBS phenotypes might be generated by defects in non-keratin genes in addition to keratin genes. The extent to which keratin gene defects are responsible for human EBS should become clearer as additional cases are examined.

It is interesting that while quite substantial K14 deletion mutations were used to generate EBS phenotypes in transgenic mice, point mutations were found in Dowling Meara EBS in humans. We have recently shown that different point mutations in K14 can perturb keratin filament networks to varying degrees, some of which can be quite severe (Letai, A., P. A. Coulombe, and E. Fuchs, manuscript submitted for publication). However, there are several lines of evidence which suggest that human epidermis may be more sensitive than mouse to K14/K5 keratin mutations. Most importantly, the abundance of hair follicles in mouse skin may both anchor and buffer the epidermis from incidental trauma. In addition, elevated expression of these genes occurs much earlier in development for humans than mouse (Kopan and Fuchs, 1989). Thus, mutations such as CΔ135K14P, which gave a high incidence of neonatal death in mice might be expected to cause embryonic lethality in humans. While further experiments will be necessary to assess the extent to which our transgenic mice provide adequate animal models for the study of human EBS, it seems clear that they have already provided valuable insights into our understanding of the molecular basis for EBS diseases. In addition, the disease has begun to provide valuable insights into our understanding of keratin function.

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