Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 3197–3201, April 1993
Medical Sciences

Disease severity correlates with position of keratin point mutations in patients with epidermolysis bullosa simplex

(keratin disorder/10-nm filament structure)

ANTHONY LETAI, PIERRE A. COULOMBE*, MARY BETH MCCORMICK, QIAN-CHUN YU, ELIZABETH HUTTON, AND ELAINE FUCHS†

Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637

Communicated by Janet D. Rowley, January 4, 1993

ABSTRACT **Keratins are the major structural proteins of the epidermis. Recently, it was discovered that point mutations in the epidermal keratins can lead to the blistering skin diseases epidermolysis bullosa simplex (EBS) and epidermolytic hyperkeratosis (EH), involving epidermal cell fragility and rupture upon mechanical stress. In this study, we demonstrate a correlation between disease severity, location of point mutations within the keratin polypeptides, and degree to which these mutations perturb keratin filament structure. Interestingly, of the 11 EBS or EH mutations thus far identified, 6 affect a single highly evolutionarily conserved arginine residue, which, when mutated, markedly perturbs keratin filament structure and keratin network formation. This site also appears to be a hot spot for mutation by CpG methylation and deamination. In the four epidermal keratins, there are several other CpG dinucleotides that exist at codons within the highly conserved ends of the keratin rod. To elucidate why mutations at these sites have not been detected in severe cases of EBS, we engineered 7 of these C → T transitions in K14 and tested their ability to perturb keratin network formation and keratin filament assembly *in vitro*. The effects of these mutants on keratin filament network formation were significantly less severe than the EBS/EH arginine mutation, suggesting that the high incidence of mutations of the residue in EBS and EH patients is a result of both a special sensitivity of filament structure to perturbations in this residue and its susceptibility to mutagenesis.**

Located at the interface between the physical and chemical traumas of the environment and the interior of the body, the epidermis serves an important protective role. It manifests this function by building an extensive cytoskeletal architecture, the unique features of which are keratin filaments. These 10-nm filaments belong to the intermediate filament (IF) superfamily (for review, see refs. 1 and 2). Keratin filaments are obligatory heteropolymers (3, 4) composed of ≈10,000 heterodimers of type I and type II proteins (5–7). Basal epidermal cells express the type II keratin K5 and the type I keratin K14 (8). As epidermal cells commit to terminal differentiation, they switch to expressing a new pair, the type II K1 and the type I K10 (9, 10).

In the past 2 years, evidence has been provided to suggest that when keratin filament formation is perturbed in epidermal cells in transgenic animals, the keratinocytes become fragile and prone to rupture upon mild physical trauma (11–13). Epidermolysis bullosa simplex (EBS) is an autosomal dominant blistering skin disease, typified by basal cell cytolysis upon mechanical stress (14). Recently, it was discovered that patients with EBS have point mutations in the coding segments of the genes encoding the basal epidermal keratins K5 and K14 (15–17). Epidermolytic hyperkeratosis (EH) is another autosomal dominant, blistering skin disease. While EH is also typified by mechanical stress-dependent cell cytolysis, it differs from EBS in that it is the suprabasal epidermal cells that exhibit cell degeneration (14). EH is also a keratin disorder, in this case involving point mutations in the differentiation-specific keratins K1 and K10 (18–20).

The secondary structures of all the epidermal keratins are similar and consist of a central, 310-amino acid residue rod domain, predicted to be largely α-helical and containing throughout a heptad repeat of hydrophobic residues, enabling the two keratins of a pair to intertwine about one another in a coiled-coil fashion (21). Using molecular mutagenesis and gene transfection, it was shown that mutations involving the rod domain are often deleterious to keratin filament network formation and 10-nm filament structure (22–28). In contrast, with the exception of a portion of the type II (K5) head domain, the head and tail domains of epidermal keratins (K5/K14) can be removed without major structural perturbations (see ref. 22 and references therein).

Thus far, characterized incidences of the most severe form of EBS, Dowling–Meara (DM), have point mutations clustered in the highly conserved ends of the K5 or K14 rod domains (15, 17), while affected members of families with severe cases of EH have point mutations in corresponding regions of the K10 and K1 rod (18, 20). In contrast, mutations in patients with milder cases of EBS and EH have mapped to less-conserved regions, either within or outside the rod domain (16, 19). Interestingly, in 6 of 11 incidences of EBS-DM or EH, point mutations were found at a highly evolutionarily conserved arginine residue in the N terminus of the K14 (EBS) or K10 (EH) rod (15, 18, 20).

Given the clustering of severe mutations in the conserved end domains of the keratin rod, we wondered whether the more severe clinical phenotypes of EBS and EH might be caused by mutations that more severely disrupt keratin filament structure. To test this hypothesis, we used site-directed mutagenesis to engineer the four known EBS mutations, K14-R125C (Arg-125 to Cys mutation), K14-R125H, K14-L384P, and K5-E475G into the appropriate cloned human wild-type K14 or K5 cDNAs. We then tested the effects of these mutations (*i*) when expressed in transfected human epidermal cells in culture and (*ii*) when subjected to 10-nm filament assembly studies *in vitro*.

Finally, we explored why the highly conserved arginine residue in the N terminus of the keratin rod, present as arginine or lysine in 50 of 51 published IF sequences, is so

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IF, intermediate filament; EBS, epidermolysis bullosa simplex; EH, epidermolytic hyperkeratosis; EBS-DM, Dowling–Meara form of EBS; EBS-K, Koebner form of EBS.

*Present address: Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.
†To whom reprint requests should be addressed at: Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, 5841 South Maryland Avenue, Room N314, Chicago, IL 60637.

frequently a target for C → T transition mutations in EBS and EH. We have engineered and tested seven additional C → T transitions at CpG dinucleotides located at conserved residues in the rod ends of epidermal keratins. Collectively, our results reveal insights into the importance of the residues known to play a role in EBS and EH and reveal a definite correlation between disease severity and the degree to which mutations lead to perturbations in 10-nm filament structure. Our results also suggest that R125 of K14 (R156 of K10) must play a special role in maintaining keratin network integrity.

MATERIALS AND METHODS

Site-Directed Mutagenesis. Point mutations were engineered in a Bluescript KS+ plasmid (Stratagene) containing the wild-type K14 or K5 cDNA as described (28). A fragment encompassing the mutation was then subcloned into either pJK14P (simian virus 40 promoter/enhancer-based mammalian expression vector; (ref. 23) or pETK14 (bacterial expression vector; ref. 24).

Transient Transfections. Epidermal cultures from EBS-DM or Koebner EBS (EBS-K) patients were fixed at 25% confluence and subjected to indirect immunofluorescence with a guinea pig antiserum against keratin 5. Human epidermal (SCC-13) keratinocytes were transiently transfected with wild-type or mutant keratin cDNAs tagged with the human neuropeptide substance P epitope and driven by the simian virus 40 major early promoter and enhancer (23). At 65 hr posttransfection, cells were fixed and examined by indirect double immunofluorescence, using a rabbit anti-substance P antiserum to recognize the neuropeptide substance P tag and the anti-K5 antiserum to recognize the endogenous keratin network. The substance P-tagged K5 cDNA is analogous to the previously described substance P-tagged K14 cDNA, with the substance P tag replacing the last 19 encoded amino acids of the K5 polypeptide. All clones were verified by sequencing the genetically engineered segments.

IF Extractions and Immunoblot Analysis. Keratins were isolated from transiently transfected cells and subjected to SDS/PAGE and immunoblot analysis as described (23), except that primary antibody incubation was followed by incubation with a horseradish peroxidase-linked protein A (Boehringer Mannheim) followed by enhanced chemiluminescent detection (Amersham) or alkaline phosphatase detection (Bio-Rad).

***In Vitro* 10-nm Filament Formation.** Wild-type and mutant K5 and K14 were combined with their wild-type partner and dialyzed against low ionic strength filament assembly buffer (24, 28). Techniques for expression of keratins in bacteria, purification by anion-exchange chromatography, and conditions for *in vitro* filament assembly have all been described (24, 28). Filaments were visualized under a Jeol electron microscope by uranyl acetate negative staining (24).

RESULTS AND DISCUSSION

Fig. 1 illustrates the location of the four EBS mutations thus far identified. The recent classification of Fine *et al.* (29) distinguishes EBS-DM from other subtypes by the prevalence of large clumps or aggregates of tonofilaments in the basal epidermal cells (14). By immunoelectron microscopy, these tonofilament clumps label with antibodies against keratins 5 and 14 (11, 30). When epidermal keratinocytes from five unrelated patients with EBS-DM were cultured, most cells at colony edges showed a withdrawal of the keratin filament network from the cytoplasmic membrane and punctate K5 or K14 staining at the cell perimeter (representative example in Fig. 2*B*, compare with network from wild-type keratinocytes in Fig. 2*A*; see also refs. 15 and 31). When the human epidermal keratinocyte line SCC-13 was transfected with EBS-DM mutants K14-R125C, K14-R125H, and K5-E475G, a pronounced collapse of the keratin network and punctate staining was seen in some of the cells that stained brightly with a transgene-specific antibody (Fig. 2 *E–G*, respectively) with frequencies of 11% ± 3% (K14-R125H), 10% ± 2% (K14-R125C), and 6% ± 2% (K5-E475G), assigning the confidence limit at 95%. These mutants behaved in a dominant-negative fashion, as judged by costaining with antibodies specific for the endogenous keratin network (data not shown; for previous double-immunofluorescence studies, see refs. 23 and 28). This phenotype was not seen in a single epidermal keratinocyte transfected with clones encoding the wild-type K14 (shown) and K5 (not shown) counterparts. This distinction was important, since in any set of keratin transfections, there are always some cells expressing high levels of wild-type transgenes that nevertheless exhibit mild perturbations and also some cells expressing low levels of severely disrupting transgenes that exhibit wild-type networks (23). In fact, >98% of the cells we transfected with the wild-type K14 or K5 counterparts exhibited a wild-type phenotype (Fig. 2*D*).

In cultures of three unrelated EBS-K cases, ≈50% of keratinocytes at colony peripheries displayed a seemingly wild-type keratin network (Fig. 2*C*). The remaining ≈50% of cells showed subtle perturbations at the cell periphery, with wisp-like, shortened filament bundles, but without the large aggregates seen in the EBS-DM keratinocytes (12). Similar to cultured EBS-K keratinocytes, most SCC-13 keratinocytes transfected with the EBS-K mutant K14-L384P exhibited few if any perturbations (e.g., see Fig. 2*H*). In fact, only a few rare (<2% with a 95% confidence limit) transfected cells displayed a keratin filament network that was unequivocally distinct from wild type (data not shown), and none of these showed the network collapse and accompanying cytoplasmic aggregates that had been seen in cells transfected with the EBS-DM mutants. The differences in phenotypes between cultures transfected with wild-type, EBS-DM, and EBS-K mutations could not be attributed to different levels of expression among the various transgenes; immunoblot anal-

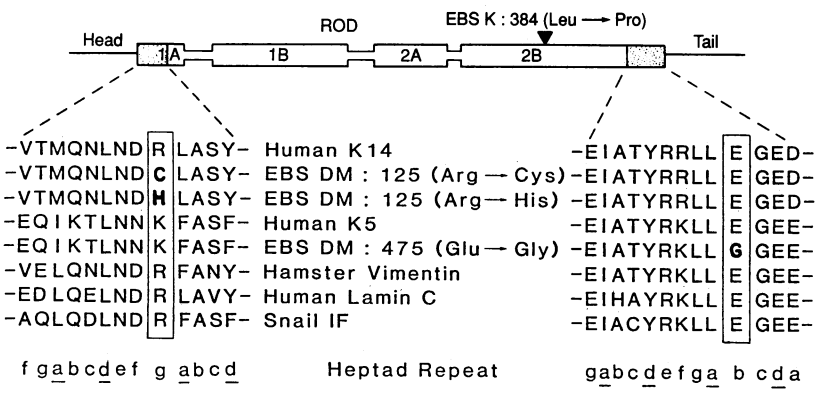

FIG. 1. Locations of human EBS mutations relative to keratin structure. Diagram of secondary structure characteristic of all IF proteins. Open boxes encompass the central 310-amino acid (356 for lamins) rod domain, predicted to be largely α-helical. Rod is subdivided into helices 1A, 1B, 2A, and 2B (2). Stippled boxes denote highly conserved end domains of the rod. Solid bars denote nonhelical head (N-terminal) and tail (C-terminal) domains. Sequences encompassing the three known EBS-DM mutations are given beneath the diagram. Shown also are corresponding sequences from more distant IF proteins. EBS-K mutation (K14-L384P) is located outside the rod ends and is indicated by the arrowhead. Numbering of residues is assuming the initiator methionine is position 1.

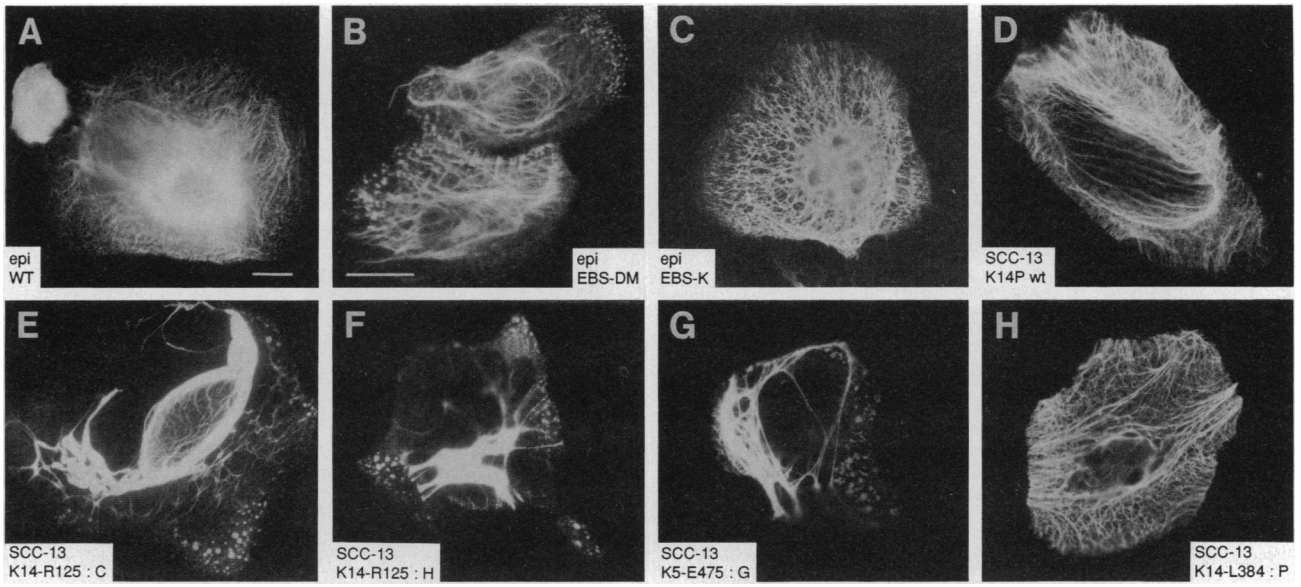

FIG. 2. Comparison of keratin networks in EBS keratinocytes and in keratinocytes transiently expressing genetically engineered EBS point mutations. Epidermal cells from six patients with EBS-DM or EBS-K were cultured as described (15). Keratin networks stained with anti-K5 antiserum are from epidermal cells of normal human skin (*A*), human EBS-DM skin (*B*), and human EBS-K skin (*C*). (*D–H*) SCC-13 keratinocytes were transfected with wild-type or mutant basal epidermal keratin cDNAs. Transfected cells were processed for double immunofluorescence by using antibodies against the transgene product (anti-substance P; shown) and against the endogenous keratin network (anti-K5; not shown). For each construct, 400–1000 transfected keratinocytes were examined and scored for whether or not they exhibited a markedly aberrant keratin filament network, with keratin aggregates at the periphery. This phenotype was chosen for scoring, because (*i*) it was not seen in keratinocytes transfected with wild-type keratin constructs, and (*ii*) it was similar to that of EBS-DM keratinocytes. Cells shown were transfected with wild-type K14P (indistinguishable from wild-type K5P; data not shown) (*D*), K14-R125C (*E*), K14-R125H (*F*), K5-E475G (*G*), and K14-L384P (*H*). Bar in *A* is for *A* and *C*; bar in *B* is for *B* and *D–H*. (Bars = 20 μm.)

yses of transfected cultures indicated that mutants were expressed at comparable levels (Fig. 3).

Collectively, these studies revealed that keratinocytes transfected with any of the three EBS-DM mutants resembled EBS-DM keratinocytes, while keratinocytes transfected with the EBS-K mutant resembled EBS-K keratinocytes. Most importantly, a correlation existed between severity of the EBS disease and the degree to which keratin filament networks were perturbed. This correlation held both for EBS keratinocytes and for keratinocytes transiently expressing a mutant EBS transgene.

To explore the parallel between clinical EBS and 10-nm filament structure, we examined the ability of bacterially expressed K14 and K5 EBS mutants to assemble into keratin filaments when combined with their wild-type partner keratin *in vitro* (Fig. 4). Under the conditions used, wild-type K5 and K14 assembled into long 10-nm filaments (Fig. 4*A*, 25 ± 10 μm). These filaments were similar in length to those obtained with keratins isolated from normal cultured keratinocytes (a mixture of roughly equal levels of K14, K5, K6, and K16; ref. 32). In contrast, filaments made from keratins isolated from EBS-DM keratinocytes were shorter (Fig. 4*B*, 0.7 ± 0.7 μm; see also ref. 15). Filaments made from genetically engineered EBS-DM mutants and their wild-type partners were also shorter and, in addition, often appeared irregular in width and partially unraveled. This phenotype was particularly noticeable for K14-R125C (Fig. 4*C*, 2 ± 2 μm) and K14-R125H (Fig. 4*D*, 1.2 ± 1.0 μm). Filaments formed with K5-E475G were nearly normal in length, but exhibited unraveling and a tendency to aggregate (Fig. 4*F*). This aggregation manifested itself as either macroscopically visible, extremely viscous droplets that were difficult to pipette; electron-opaque balls, visible by EM; or poorly dispersed groups of filaments. In contrast, filaments formed with K14-L384P were shorter than wild-type (9 ± 4 μm) but showed little evidence of fraying (Fig. 4*E*). Filaments assembled from keratins isolated from EBS-K keratinocytes were also only slightly different from wild type (P.A.C. and E.F., unpublished data). Thus, the characteristics of keratin filaments assembled from each of four EBS mutants and their wild-type partners deviated from wild-type 10-nm filaments, and a rough correlation existed between the degree to which filament assembly was perturbed *in vitro* and the degree with which these mutants altered keratin networks in transfected keratinocytes. Collectively, our data support the notion that the position of the point mutation in the keratin is of primary importance in determining the severity of the disease EBS *in vivo*.

Our studies lead us to predict that a mutation in K5 or K14 that causes (*i*) appreciable network collapse and peripheral tonofilament clumping in transiently transfected keratinocytes and (*ii*) perturbations in 10-nm filament assembly and structure *in vitro* is very likely to cause the DM subtype of EBS if found in a human allele. In contrast, more subtle rod mutations, including those within and outside the ends, may be expected to generate a more subtle clinical phenotype, as indicated by the L384P studies. These findings are in good agreement with our prior mutagenesis studies, which illustrated that even subtle mutations in the ends of the rod domain were more critical to 10-nm filament structure than proline substitutions in the central portion of the rod (28). Given the subtle *in vitro* behavior of the L384P mutation (this study) and other proline mutations that fall outside of the rod end domains (28), it may be difficult to investigate the mildest cases of EBS by the functional assays described here.

Based on point mutagenesis studies on IF proteins, we would have predicted that at least 20 different residues in the K14 and K5 rod ends could yield a DM phenotype (26, 28, 33). Thus, it was striking that two EBS-DM mutations occurred at the same residue, K14-R125 (15), and we have now found a second case of K14-R125C in an unrelated EBS-DM patient (data not shown). In addition, the Arg to His mutation has now been found in the corresponding position of K10 in three of six characterized incidences of the related skin disease EH (18–20).

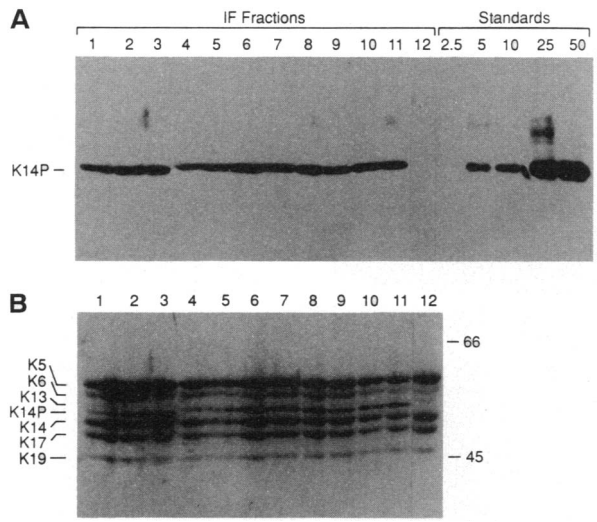

FIG. 3. Mutant keratins in transfected keratinocytes are expressed at comparable levels. SCC-13 keratinocytes (100-mm dishes) were transfected with each of the keratin mammalian expression constructs discussed. At 65 hr posttransfection, cells were harvested and IF proteins were extracted. Proteins were resolved by electrophoresis through SDS/8.5% polyacrylamide gels, followed by transfer to an Immobilon-P poly(vinylidene difluoride) membrane (Millipore). The blot was subjected to immunoblot analysis with a rabbit polyclonal anti-substance P antiserum (1:1000 dilution), detected by enhanced chemiluminescence (*A*) and with detection of total protein by Coomassie blue staining to demonstrate equal loading and transfer (*B*). In *B*, K14P has been visualized by alkaline phosphatase methods to demonstrate its mobility relative to wild-type keratins. Bacterially expressed K5 and K14P, twice purified by anion-exchange chromatography, were used as mass and immunoblot standards. On the right, K14P standards are shown at 2.5, 5, 10, 25, and 50 ng per band. Lanes 1–12 contain 5-μg aliquots of IF proteins extracted from cells transfected as follows: lane 1, K14P; lane 2, R125C; lane 3, R125H; lane 4, L384P; lane 5, R134C; lane 6, R134H; lane 7, R134Q; lane 8, R134W; lane 9, R416C; lane 10, R416H; lane 11, E422K; lane 12, no DNA. Mass standards in kDa at right. Amounts of total K14 (endogenous) and K14P (transgene product) were estimated by comparison with mass standards on Coomassie-stained SDS/polyacrylamide gel (on a separate gel; data not shown) and immunoblot (*A*), respectively. K14P (ng) was divided by total K14 protein (ng) on the immunoblot; this number was divided by the proportion of transfected cells (i.e., anti-substance P positive) in the population to give the average ratio of transgene K14P to K14 per transfected cell. It was estimated that the mutant and wild-type K14P constructs expressed at an average level of 30–60% of endogenous K14 levels. Similar immunoblot analysis with K5P and K5-E475G revealed that these two transgenes were expressed at 10–20% of endogenous K5 levels. Western blot analysis was necessary to quantitate transgene products because low (1–3%) transfection efficiencies yielded insufficient material for Coomassie staining.

This arginine residue is encoded by CGC. CpG sequences are underrepresented 3- to 5-fold in vertebrate genomes (34, 35), suggesting a net loss of these dinucleotides during evolution. Nevertheless, they account for a disproportionately high number (>30%) of mutations in human genetic disorders that involve point mutations (36). The underlying basis for the high frequency of CpG mutations almost certainly resides in methylation of CpG dinucleotides at the 5′ position of cytosine followed by deamination (37). This reaction gives rise to a C → T transition, which, in the coding strand of a CGC codon, yields an Arg to Cys mutation and, in the noncoding strand, an Arg to His mutation.

There are a number of other CpG dinucleotides in the K14 and K5 coding sequences. In some cases, the C is in the third codon position and would not change the amino acid if mutated. In addition, a number of other CpG dinucleotides exist either in the nonhelical domains or in the interior of the rod. Much of these regions might be expected to tolerate point mutations without severe consequence to filament structure (see, e.g., ref. 28). Those CpG sequences that do reside in conserved residues within the rod ends—i.e., residues that, if mutated, might be expected to generate an EBS-DM phenotype—are K5-D184, K14-R125 (K5-R187), K14-R134, K5-A468, K14-R416 (K5-R471), K14-R417, and K14-E422 (K5-E477). It is surprising that of these, only the K14-R125 (K10-R156) has been found in EBS and EH patients, and, furthermore, it has been detected at high frequency (6 of 11 published cases). That mutations in the other residues have not been found in EBS-DM or severe EH could mean either that these mutations cause more subtle perturbations, that they are lethal, or that these sites are not naturally methylated in the genome.

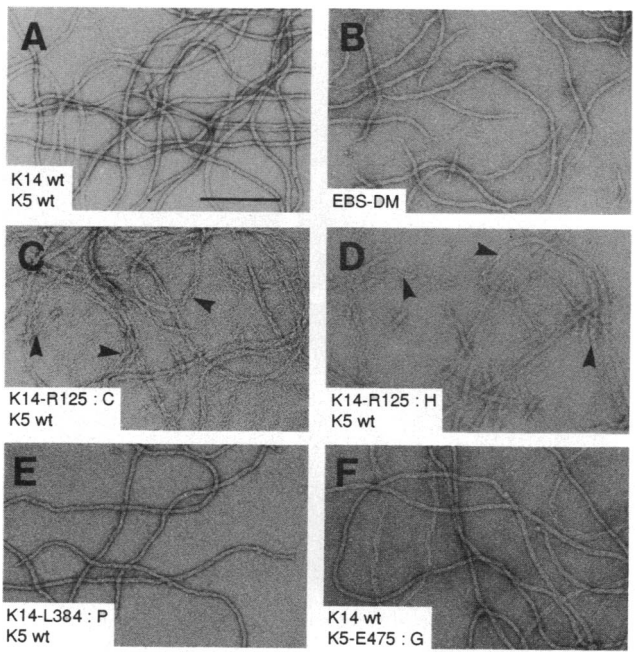

FIG. 4. *In vitro* filament assembly of keratins containing point mutations found in EBS patients. Keratins were isolated and subjected to 10-nm filament assembly *in vitro* as described. Shown are filaments formed from wild-type K5 and K14 (*A*), keratin proteins isolated from epidermal cells of a patient with EBS-DM (*B*), K14-R125C and wild-type K5 (*C*), K14-R125H and wild-type K5 (*D*), K14-L384P and wild-type K5 (*E*), wild-type K14 and K5-E475G (*F*). Arrowheads in *C* and *D* indicate regions of unraveling. (Bar = 200 nm.)

To ascertain whether these mutations might cause less or more severe phenotypes than K14-R125 (K10-R156), we constructed the following mutations in K14: R134C, R134H, R134Q, R134W, R416C, R416H, and E422K (Fig. 5). These mutations could occur via methylation/deamination at conserved CpG dinucleotides in the encoded rod ends. R134C and R134H represent mutation of the CGC codon found in K14, while R134Q and R134W represent mutation of the corresponding CGG codon found in K10. These mutations were subcloned into our mammalian expression vector and expressed in SCC-13 cells by transient transfection.

In all seven cases, the observed phenotype was less severe than that found in cells transfected with R125 mutants (compare representative cells in Fig. 5 *A–C* with that in Fig. 2*E*). Of these seven CpG mutants, only very rare cells (<1% with a 95% confidence limit) exhibited a network collapse and peripheral keratin clumping that was observed in the R125 mutants. Given these findings, it seems unlikely that these C → T transitions at CpG dinucleotides will surface in the pool of keratin mutations that give rise to EBS-DM or severe EH.

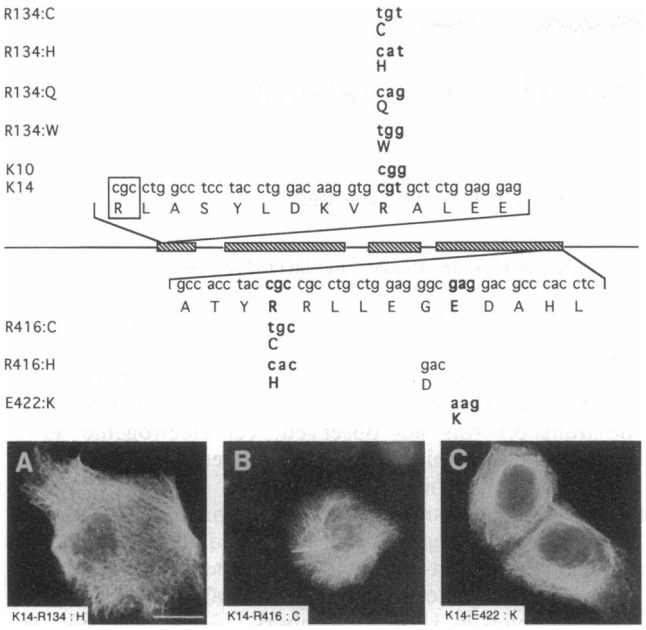

FIG. 5. Characterization of additional rod end mutants involving CpG dinucleotides. Diagram represents location of residues R134, R416, and E422, with R125 boxed for comparison. Shown are nucleotide sequences of the rod ends and the C → T point mutations at CpG dinucleotides that were engineered into the wild-type K14 cDNA. K10 codon is shown for the residue corresponding to K14-R134. Note that R416H is in fact a double mutant, containing also the mutation G421D. SCC-13 keratinocytes were transfected with each of these CpG mutants, and, at 65 hr posttransfection, cells were fixed and stained with a rabbit antiserum against the substance P tag, followed by fluorescein isothiocyanate-conjugated goat anti-rabbit secondary antiserum. Shown are representative examples of transfected SCC-13 cells expressing R134H (*A*), R416C (*B*), and E422K (*C*). All the other mutants also gave a similar wild-type phenotype. Note that the wild-type phenotype of these seven CpG mutations was in striking contrast to R125C or R125H. (Bar = 20 μm.)

At present, our methods of assay are not sufficiently sensitive to assess whether these mutations might arise in milder EBS or EH cases. However, based on the knowledge that 3 of 75 published IF sequences have the natural equivalent of the E422K change, 1 of 54 IF sequences has the natural equivalent of R134Q, and 8 of 54 IF sequences have the natural equivalent of R134H, these changes appear to be tolerated naturally in at least some IF proteins.

In summary, our results suggest that the reason why K14-R125 and K10-R156 residues are mutated at high frequency in severe cases of EBS and EH is that this is one of only a very few, and possibly the only, codons that both contain CpG dinucleotides and also have a special importance to 10-nm keratin filament assembly. Furthermore, the presence of an arginine analogous to K14-R125 and K10-R156 in almost all IF proteins suggests that this residue is a likely candidate for mutagenesis in other as yet unidentified degenerative diseases that might have as their genetic bases point mutations in one of the many other IF proteins.

We have also provided evidence that in EBS, and most likely EH as well, clinical manifestations of the disease correlate with the extent to which point mutations interfere with keratin filament formation. Coupled with previous studies, our data suggest that the most severe cases of EBS and EH will correlate with point mutations occurring within the highly conserved rod ends of the major epidermal keratins. Finally, our results suggest that keratin mutants causing even very subtle changes in 10-nm filaments *in vitro* can nevertheless cause alterations in the mechanical integrity of epidermal cells, leading to blistering on a macroscopic scale. These studies have broad implications for appreciating the subtleties in architectural perturbations that can lead to loss of tissue function in the development of human diseases involving structural proteins.

We thank Drew Syder for his assistance in sequencing engineered keratin mutants, Grazina Traska for her assistance in tissue culture, and Philip Galiga for artwork. We thank Dr. Amy S. Paller (Northwestern University Medical School, Chicago), Dr. Adelaide Hebert (University of Texas Health Science Center, Houston), and Dr. Roger Pearson (Rush Medical School, Chicago) for the EBS patient biopsies used for cell culture. This work was supported by a grant from the National Institutes of Health (AR27883). E.F. is an investigator of the Howard Hughes Medical Institute.

1. Albers, K. & Fuchs, E. (1992) *Int. Rev. Cytol.* **134,** 243–279.
2. Conway, J. F. & Parry, D. A. D. (1988) *Int. J. Biol. Macromol.* **10,** 79–98.
3. Steinert, P. M., Idler, W. W. & Zimmermann, S. B. (1976) *J. Mol. Biol.* **108,** 547–567.
4. Franke, W. W., Schiller, D. L., Hatzfeld, M. & Winter, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7113–7117.
5. Coulombe, P. & Fuchs, E. (1990) *J. Cell Biol.* **111,** 153–169.
6. Hatzfeld, M. & Weber, K. (1990) *J. Cell Biol.* **110,** 1199–1210.
7. Steinert, P. M. (1990) *J. Biol. Chem.* **265,** 8766–8774.
8. Nelson, W. & Sun, T.-T. (1983) *J. Cell Biol.* **97,** 244–251.
9. Fuchs, E. & Green, H. (1980) *Cell* **19,** 1033–1042.
10. Roop, D. R., Huitfeldt, H., Kilkenny, A. & Yuspa, S. H. (1987) *Differentiation* **35,** 143–150.
11. Vassar, R., Coulombe, P. A., Degenstein, L., Albers, K. & Fuchs, E. (1991) *Cell* **64,** 365–380.
12. Coulombe, P. A., Hutton, M. E., Vassar, R. & Fuchs, E. (1991) *J. Cell Biol.* **115,** 1661–1674.
13. Fuchs, E., Esteves, R. A. & Coulombe, P. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6906–6910.
14. Anton-Lamprecht, I. (1983) *J. Invest. Dermatol.* **81,** Suppl. 1, 149s–156s.
15. Coulombe, P. A., Hutton, M. E., Letai, A., Hebert, A., Paller, A. S. & Fuchs, E. (1991) *Cell* **66,** 1301–1311.
16. Bonifas, J. M., Rothman, A. L. & Epstein, E. H. (1991) *Science* **254,** 1202–1205.
17. Lane, E. B., Rugg, E. L., Navsaria, H., Leigh, I. M., Heagerty, A. H. M., Ishida-Yamamoto, A. & Eady, R. A. J. (1992) *Nature (London)* **356,** 244–246.
18. Cheng, J., Syder, A., Letai, A., Paller, A. S. & Fuchs, E. (1992) *Cell* **70,** 811–819.
19. Chipev, C. C., Korge, B. P., Markova, N., Bale, S. J., DiGiovanna, J. J., Compton, J. G. & Steinert, P. M. (1992) *Cell* **70,** 821–828.
20. Rothnagel, J. A., Dominey, A. M., Dempsey, L. D., Longley, M. A., Greenhalgh, D. A., Gagne, T. A., Huber, M., Frenk, E., Hohl, D. & Roop, D. R. (1992) *Science* **257,** 1128–1130.
21. Hanukoglu, I. & Fuchs, E. (1983) *Cell* **33,** 915–924.
22. Wilson, A. K., Coulombe, P. A. & Fuchs, E. (1992) *J. Cell Biol.* **119,** 401–414.
23. Albers, K. & Fuchs, E. (1987) *J. Cell Biol.* **105,** 791–806.
24. Coulombe, P., Chan, Y. M., Albers, K. & Fuchs, E. (1990) *J. Cell Biol.* **111,** 3049–3064.
25. Lu, X. & Lane, E. B. (1990) *Cell* **62,** 681–696.
26. Hatzfeld, M. & Weber, K. (1991) *J. Cell Sci.* **99,** 351–362.
27. Bader, B. L., Magin, T. M., Freundenmann, M., Stumpp, S. & Franke, W. W. (1991) *J. Cell Biol.* **115,** 1293–1307.
28. Letai, A., Coulombe, P. & Fuchs, E. (1992) *J. Cell Biol.* **116,** 1181–1195.
29. Fine, J.-D., Bauer, E. A., Briggaman, R. A., Carter, D.-M., Eady, R. A. J., Esterly, N. B., Holbrook, K. A., Hurwitz, S., Johnson, L., Lin, A., Pearson, R. & Sybert, V. P. (1991) *J. Am. Acad. Dermatol.* **24,** 119–135.
30. Ishida-Yamamoto, A., McGrath, J. A., Chapman, S. J., Leigh, I. M., Lane, E. B. & Eady, R. A. J. (1991) *J. Invest. Dermatol.* **97,** 959–968.
31. Kitajima, Y., Inoue, S. & Yaoita, H. (1989) *Arch. Dermatol. Res.* **281,** 5–10.
32. Sun, T.-T. & Green, H. (1978) *J. Biol. Chem.* **253,** 2053–2060.
33. Heald, R. & McKeon, F. (1990) *Cell* **61,** 579–589.
34. Grippo, P., Iaccarino, M., Parisi, E. & Scarano, E. (1968) *J. Mol. Biol.* **36,** 195–208.
35. Nussinov, R. (1981) *J. Mol. Biol.* **149,** 125–131.
36. Cooper, D. N. & Youssoufian, H. (1988) *Hum. Genet.* **78,** 151–155.
37. Shen, J.-C., Rideout, W. M. & Jones, P. A. (1992) *Cell* **71,** 1073–1080.