Type VII Collagen Gene Expression by Cultured Human Cells and in Fetal Skin

Abundant mRNA and Protein Levels in Epidermal Keratinocytes

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Abstract

Type VII collagen, a genetically distinct member of the collagen family, is present in the cutaneous basement membrane zone as an integral component of the anchoring fibrils. We have recently isolated several cDNAs that correspond to human type VII collagen sequences. One of these cDNAs (clone K-131) was utilized to examine type VII collagen gene expression in cultures of human cells by Northern analyses, in situ hybridizations and indirect immunofluorescence. Northern hybridizations revealed the presence of an ~ 9-kb mRNA transcript, and indicated a high level of expression in epidermal keratinocytes as well as in an oral epidermoid carcinoma cell line (KB), while the expression was considerably lower in skin fibroblasts and in several virally or spontaneously transformed epithelial cell lines. In situ hybridizations of cultured keratinocytes supported the notion of a high level of gene expression. Indirect immunofluorescence of skin from a 19-wk fetus revealed type VII collagen gene expression at the dermal-epidermal basement membrane zone. These results indicate that several different cell types including epidermal keratinocytes and dermal fibroblasts express the type VII collagen gene, but epidermal keratinocytes may be the primary cell source of type VII collagen in developing human skin. (*J. Clin. Invest.* 1992. 89:163–168.)
Key words: cutaneous basement membrane zone • anchoring fibrils • dystrophic epidermolysis bullosa

Introduction

The collagens comprise a family of closely related, yet genetically distinct macromolecules, and currently at least 15 different vertebrate collagens have been identified (for review on collagens, see refs. 1, 2). All collagen molecules consist of three polypeptide subunits, known as α-chains, and there are as many as 25 different genes within the human genome that encode these polypeptides (1, 2). The genetically distinct collagens have characteristic tissue distributions. For example, the more abundant collagens, such as type I and type III collagens, are widely distributed, while certain minor collagens have a restricted anatomical location. Among the minor collagens is type VII collagen, which is found in the basement membrane zone beneath the stratified squamous epithelium (3). Specifically, type VII collagen has been demonstrated by immunolocalization studies to be a component of anchoring fibrils, morphologically distinct structures extending perpendicularly from the lamina densa to the upper papillary dermis (4, 5).

Type VII collagen is a homotrimer, $[\alpha 1(VII)]_3$, and each α-chain consists of a central collagenous domain of ~ 145 kD that is flanked by noncollagenous domains (3, 5). The tissue form of type VII collagen has been suggested to be an antiparallel dimer associated through an overlap region between the individual molecules (6). These antiparallel dimers then aggregate laterally to form anchoring fibrils that can be recognized in transmission electron microscopy of the skin by their characteristic banding pattern (7, 8).

The precise cellular origin of type VII collagen, which comprises the anchoring fibrils, is not known. Previous studies utilizing indirect immunofluorescence and immunoprecipitation with type VII collagen–specific antibodies have suggested that both fibroblasts and keratinocytes, the two principal cell types in the skin, synthesize type VII collagen (9, 10). However, elucidation of type VII collagen gene expression at the mRNA level has been hampered by the lack of availability of corresponding DNA clones.

We have recently isolated cDNA clones that correspond to human type VII collagen sequences (11). In this study, we utilized one of these cDNAs to examine type VII collagen gene expression in cultured human cells, including epidermal keratinocytes and dermal fibroblasts, as well as several spontaneously or virally transformed cell lines. Furthermore, we have examined type VII collagen gene expression in fetal human skin by indirect immunofluorescence. The results demonstrate the expression of the type VII collagen gene in keratinocytes and fibroblasts in vitro, however, the epidermal keratinocytes may be the major source of type VII collagen in vivo.

Methods

Cell cultures and skin specimens. Human adult epidermal keratinocytes were obtained from Clonetics Corp. (San Diego, CA). The cultures were maintained in serum-free, low calcium (0.15 mM) keratinocyte growth medium supplemented with epidermal growth factor, hydrocortisone, insulin, and bovine pituitary extract (KGM, Clonetics Corp.) (12). Human skin fibroblast strains, obtained from the American Type Culture Collection (Rockville, MD) or the Human Genetic Mutant Cell Repository (Camden, NJ), were cultured in DME supplemented with 2 mM glutamine, 50 μg/ml streptomycin, 200 U/ml penicillin-G, 20 mM *N*-2-hydroxyethyl-piperazine-2′-ethane sulfonic acid, pH 7.4, and 10% FCS (13). A human oral epidermoid carcinoma KB cell line (14) and a human amniotic epithelial WISH cell line (15) were obtained from the American Type Culture Collection. Human keratinocytes transformed either with human papilloma virus (cell line HPK) or with *ras* oncogene (RHEK) were maintained in DME supplemented with 10% FCS (16). Cocultures of epidermal keratinocytes and skin fibroblasts were established by seeding these cells in 4-cm^2 chamber slides in a 10:1 ratio (10^4 keratinocytes and 10^3 fibroblasts).

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Skin biopsy specimens were obtained from a normal 19-wk fetus and immediately frozen in liquid nitrogen.

Isolation of RNA and Northern hybridizations. Total RNA was isolated from cell cultures by a single-step method, as described previously (17). RNA samples were separated on 0.9% agarose gels containing 2.2 M formaldehyde (18), transferred to Zeta probe nylon filters (BioRad Laboratories, Richmond, CA) by vacuum transfer (VacuGene, XL; LKB, Bromma, Sweden), and immobilized by heating at 80°C for 30 min. The filters were prehybridized and hybridized with cDNAs radiolabeled by the random priming method using [^{32}P]dCTP (19). After hybridization at 42°C, the filters were washed to a final stringency of 0.1× SSC/0.1% SDS at 65°C when hybridized with the human probes or at 55°C when hybridized with the rat GAPDH cDNA. The radioactive cDNA-mRNA hybrids were visualized by autoradiography by exposure of the filters to x-ray films (X-Omat, Eastman Kodak Co., Rochester, NY).

The following cDNAs were used for Northern hybridizations: For detection of type VII collagen mRNA, a 1.9 kb cDNA (K-131) was isolated from a keratinocyte λgt11 cDNA expression library by immunoscreening with the IgG fraction of circulating serum antibodies in a patient with acquired epidermolysis bullosa (11). This cDNA has been shown to correspond to human type VII collagen sequences, and the details are reported elsewhere (11). In some experiments, the radioactive type VII collagen cDNA was removed by boiling the filters twice in 0.1× SSC/0.5% SDS for 10 min. Filters were then prehybridized and rehybridized with a 1.8 kb human proα1(I) collagen (20) or with a human 2.3-kb bullous pemphigoid antigen (BPAG1) (21) cDNA. All filters were also rehybridized with a cDNA for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (22).

In situ hybridizations. Cultured cells were fixed and permeabilized in 100% ethanol for 15 min at −20°C, followed by fixation in 4% paraformaldehyde in PBS at room temperature. The cell preparations were then pretreated as described previously (23, 24). Before hybridizations, the samples were heated at 90°C for 5 min and cooled rapidly on ice. Hybridizations were then carried out in a solution containing 50% formamide, 10 mM DTT, 1 mg/ml salmon sperm DNA, 1 mg/ml baker's yeast tRNA, 2 mg/ml BSA, and a labeled cRNA probe (see below) in 2× SSC, for 3 h at 52°C. After hybridization, the specimens were incubated with 50% formamide in 4× SSC and treated with RNaseA (100 μg/ml; Sigma Chemical Co., St. Louis, MO) and RNase T1 (1 μg/ml; Boehringer-Mannheim Diagnostics, Inc., Houston, TX) in 2× SSC with intermittent washes in 2× SSC. The specimens were then dehydrated in ethanol and air dried. The ^{32}P-cRNA-mRNA hybrids were detected by immersing the samples into autoradiography emulsion (NTB-3; Eastman Kodak), diluted with an equal volume of 0.6 M ammonium acetate, and exposing them in a desiccant-containing box for 10–12 d at 4°C. The samples were developed with D-19 developer (Eastman Kodak), stained with hematoxylin, dehydrated with ethanol, cleared in xylene, and mounted.

The type VII collagen cRNA probe was generated from the linearized K-131 clone utilizing a commercial RNA transcription kit (Stratagene Inc., La Jolla, CA). The transcribed RNA was purified by phenol/chloroform extraction, precipitated with 100% ethanol and resuspended in distilled water treated with diethyl pyrocarbonate.

Indirect immunofluorescence. Cultured cells on chamber slides were fixed in cold (−20°C) ethanol, or 5-μm-thick frozen tissue sections were rinsed with Tris-buffered saline (TBS, pH 7.6) and preincubated for 60 min with TBS containing 1% BSA. The samples were exposed overnight at 4°C to a monoclonal anti-type VII collagen antibody L_3D (kindly provided by Dr. David Woodley) (25). The sections were washed in TBS for 60 min with five changes and incubated with fluorescein isothiocyanate (FITC)-conjugated or with tetramethylrhodamine isothiocyanate (TRITC)-conjugated secondary antibodies (Miles Laboratories, Inc., Elkhardt, IN). After a 60-min incubation at room temperature, the sections were washed with TBS for 60 min, rinsed with distilled water, and examined with a fluorescence microscope equipped with filters for detection of FITC and TRITC.

Results

Detection of type VII collagen mRNA transcripts in cultured cells by Northern analyses. Northern hybridizations with RNA isolated from cultured human cells were performed with a recently developed human type VII collagen cDNA, K-131. As reported elsewhere (11), this 1.9-kb cDNA encodes a protein with epitopes that are recognized by antibodies to type VII collagen in Western immunoblot analysis. The protein segment encoded by K-131 consists of a carboxyl-terminal region characterized by repeating glycine-X-Y triplets, as deduced from nucleotide sequences (Fig. 1). The 5′ end of the clone encodes a noncollagenous domain, and the junction between the noncollagenous and collagenous segments contains a deduced amino acid sequence that has a high degree of homology with a peptide sequence found in type VII collagen (11, 26). These features unequivocally identified the clone K-131 as a human type VII collagen cDNA (11).

Hybridization of total RNA from cultured keratinocytes with the type VII collagen cDNA revealed a single mRNA (Fig. 2 *A*, lane *a*), and an mRNA transcript of the same size was detected with RNA isolated from cultured skin fibroblasts (lane *d*) or HPK cells (lane *c*), while no hybridization signal could be detected with RNA from RHEK cells (lane *b*) when examined in parallel (Fig. 2 *A*). In an independent experiment (Fig. 2 *C*), Northern analysis of RNA isolated from WISH cells (lane *a*) or KB cells (lane *b*) revealed a clearly detectable signal, but the mRNA abundance was lower in WISH cells. The differences in type VII collagen mRNA levels were not due to uneven loading of RNA, because hybridization of the same filters with a GAPDH cDNA yielded a signal of approximately the same intensity in all lanes (Fig. 2 *B* and *C*). Thus, human keratinocytes and skin fibroblasts actively express the type VII collagen gene. Also, KB cells contained a relatively high abundance of type VII collagen mRNAs, while a lower level of expression could be detected in other transformed cell lines.

The size of the type VII collagen mRNA, ~ 9 kb, was estimated from the relative mobilities of the mRNA for 230-kD bullous pemphigoid antigen (~ 9 kb; ref. 21) in keratinocytes (lane *a*) or the two transcripts for human proα1(I) collagen (5.8 and 4.8 kb; ref. 27) in fibroblasts (lane *d*) when the original filters were rehybridized with the corresponding cDNAs (Fig. 2 *A*).

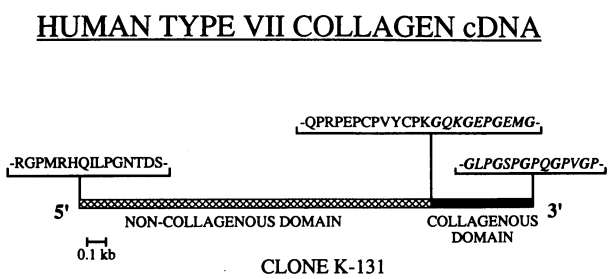

Figure 1. Schematic representation of the human type VII collagen cDNA clone K-131. This 1.9-kb cDNA consists of a noncollagenous (▨) and a collagenous (■) domain. The amino acid sequences deduced from nucleotide sequences at the 5′ and 3′ ends of the clone, as well as at the junction between the noncollagenous and collagenous domains, are indicated. Note the presence of glycine (*G*) in every third position in the collagenous portion of the molecule.

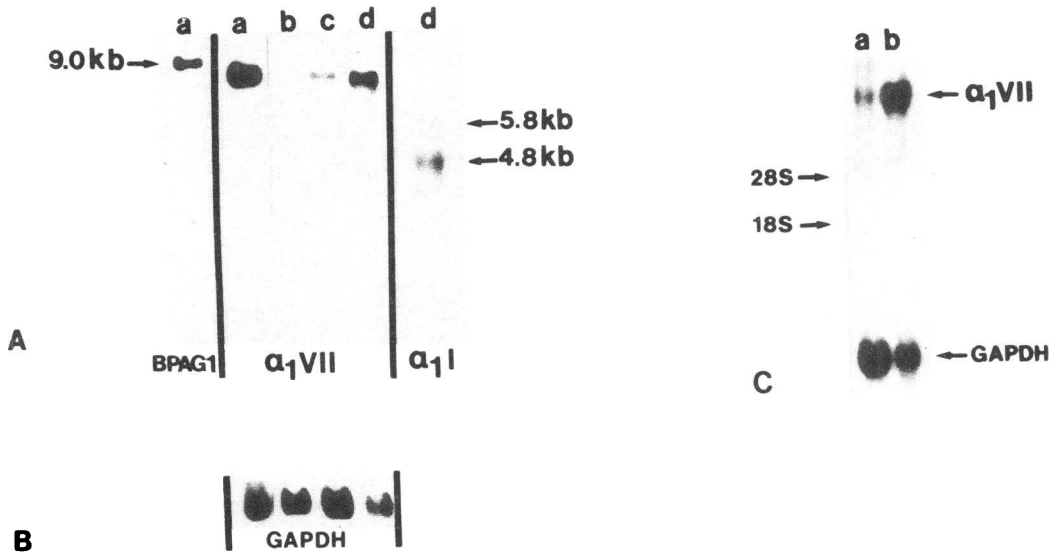

Figure 2. Detection of type VII collagen mRNAs by Northern hybridizations with the cDNA clone K-131. (*A*) Total RNA (30 μg/lane) isolated from cultures of epidermal keratinocytes (lane *a*), *ras* oncogene transformed human epidermal keratinocytes (RHEK, lane *b*), human papilloma virus transformed epidermal keratinocytes (HPK, lane *c*), or skin fibroblasts (lane *d*) was fractionated on 0.9% agarose gel, transferred to nylon filter, and hybridized first with the human type VII collagen cDNA (*middle panel*). Subsequently, the RNA in lane *a* was rehybridized with a cDNA corresponding to human bullous pemphigoid antigen-1 (230-kD protein) that recognizes an ~ 9.0-kb mRNA (*left panel*). The RNA in lane *d* was rehybridized with a proα1 (I) collagen cDNA that recognizes two transcripts of 5.8 and 4.8 kb, respectively (*right panel*). In comparison to these markers, the size of type VII collagen mRNAs is ~ 9 kb. (*B*) Rehybridization of the same filter with a GAPDH cDNA indicates that there are relatively small differences in the loading of the total RNA in lanes *a–d*. (*C*) Total RNA (30 μg/lane) isolated from WISH cells (lane *a*) or KB cells (lane *b*) also revealed the presence of type VII collagen mRNAs when hybridized with the K-131 cDNA. Rehybridization with the GAPDH cDNA served as an interval control for RNA loading.

Detection of type VII collagen mRNAs in keratinocytes by in situ hybridization. The expression of the type VII collagen gene in keratinocytes was also verified by in situ hybridizations of cultured cells. Specifically, an abundance of grains was found to be topographically localized within the cytoplasm of keratinocytes (Fig. 3 *A*). When co-cultures containing a mixture of keratinocytes and fibroblasts were examined, individual fibroblasts, distinguished from keratinocytes by their elongated, spindle-shaped appearance in phase contrast microscope, contained few, if any, autoradiographic grains suggesting a low level of expression (Fig. 3 *B*). However, keratinocytes, particularly when in close cell–cell contact, appeared to express relatively high levels of type VII collagen mRNAs (Fig. 3 *B*).

Indirect immunofluorescence of cultured cells with anti–type VII collagen antibodies. To examine type VII collagen gene expression by cultured cells at the protein level, indirect immunofluorescence with a monoclonal anti–type VII collagen antibody (25) was performed. Staining of keratinocyte cultures revealed a clearly detectable immunofluorescence signal in the cytoplasm of individual keratinocytes in a granular staining pattern (Fig. 4 *A*). Parallel staining of skin fibroblast cultures revealed a significantly lower, yet clearly detectable, immunofluorescence signal, suggesting that type VII collagen is synthesized by fibroblasts at a relatively low level (Fig. 4 *B*). Incubation of keratinocytes and fibroblasts in co-cultures revealed that the individual cells expressed enhanced levels of type VII collagen epitopes, as judged by parallel staining of cultures containing keratinocytes or fibroblasts alone (see Fig. 4 *C* in comparison to Figs. 4 *A* and *B*). These observations suggest that cell–cell interactions may play a role in enhancing the expression of type VII collagen epitopes in these cells.

Indirect immunofluorescence was also performed on WISH and KB cells in culture. Both of these cell lines demonstrated clearly detectable immunofluorescence, suggesting synthesis of type VII collagen in these cells (Figs. 4 *D* and *E*). However, in parallel with the mRNA levels, the signal observed in KB cell cultures was significantly higher than in WISH cells when these two cell types were examined in parallel.

Type VII collagen gene expression in fetal skin. Previous studies have demonstrated that type VII collagen epitopes are present in the adult human skin at the dermal–epidermal basement membrane zone (28, 29). In this study, skin from a 19-wk fetus was examined by indirect immunofluorescence, which demonstrated the presence of type VII collagen epitopes in a linear pattern (Fig. 5). No immunosignal could be detected in the more superficial layers of epidermis or within the dermis. Thus, basal keratinocytes appear to be the primary source of type VII collagen in developing fetal skin.

Discussion

Type VII collagen, a relatively low abundance protein among the collagenous molecules, is an integral component of anchoring fibrils (3–5). Previous characterization of type VII collagen by biochemical and immunochemical means has suggested that type VII collagen molecules consist of three identical α-chains, α1(VII) (3). Each α-chain is thought to contain a large central collagenous domain flanked by noncollagenous segments at both the amino- and carboxyl-terminal ends. Previous studies have suggested that the large noncollagenous globular domain (NC-1) of the type VII collagen molecule resides in the carboxylterminus (3, 5). However, recent cloning of type VII collagen sequences has provided evidence that NC-1 may in fact be located at the amino-terminus, and the carboxyl-terminal domain (NC-2) is relatively small (11, and A. Christiano, unpublished).

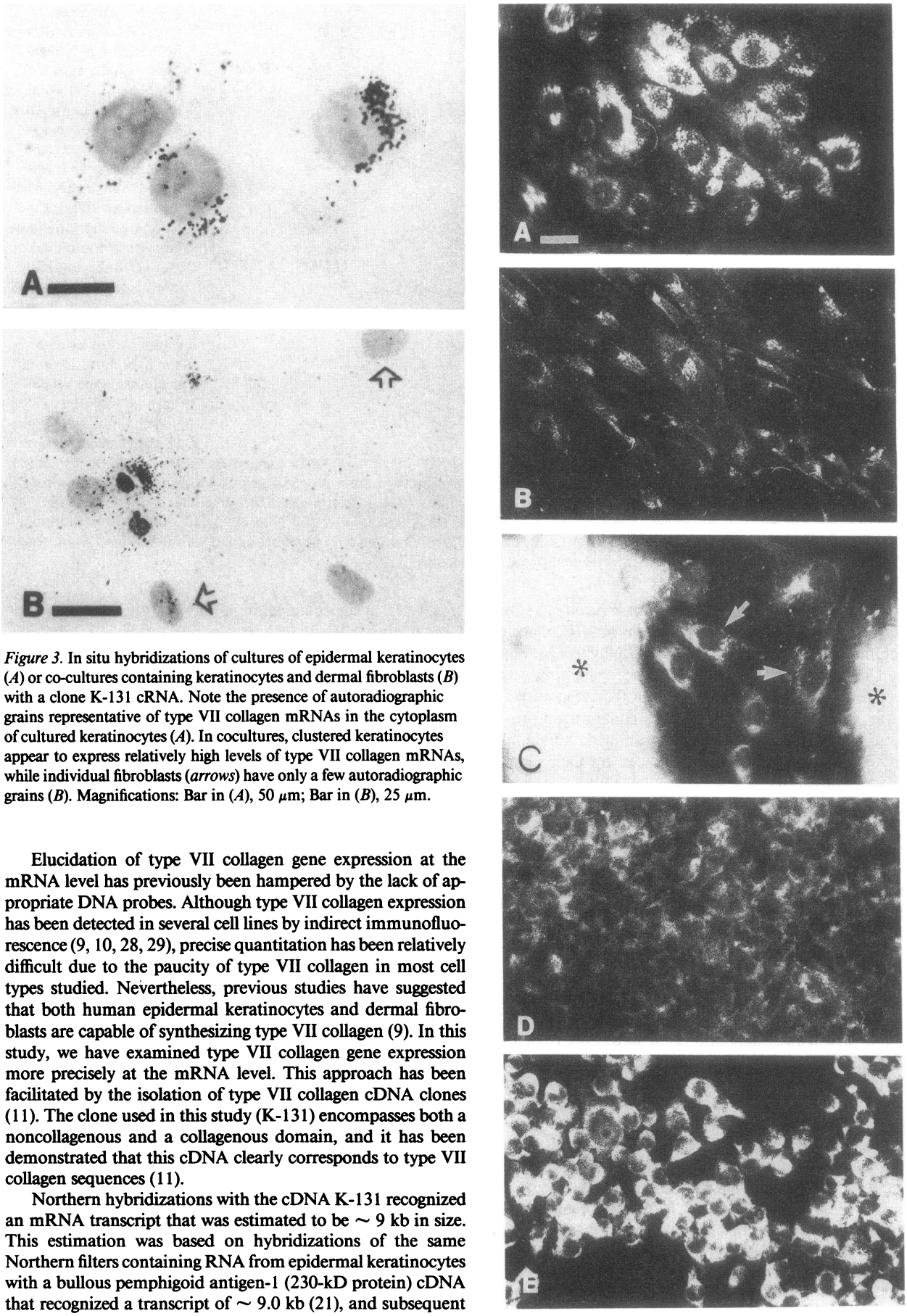

Figure 3. In situ hybridizations of cultures of epidermal keratinocytes (*A*) or co-cultures containing keratinocytes and dermal fibroblasts (*B*) with a clone K-131 cRNA. Note the presence of autoradiographic grains representative of type VII collagen mRNAs in the cytoplasm of cultured keratinocytes (*A*). In cocultures, clustered keratinocytes appear to express relatively high levels of type VII collagen mRNAs, while individual fibroblasts (*arrows*) have only a few autoradiographic grains (*B*). Magnifications: Bar in (*A*), 50 μm; Bar in (*B*), 25 μm.

Elucidation of type VII collagen gene expression at the mRNA level has previously been hampered by the lack of appropriate DNA probes. Although type VII collagen expression has been detected in several cell lines by indirect immunofluorescence (9, 10, 28, 29), precise quantitation has been relatively difficult due to the paucity of type VII collagen in most cell types studied. Nevertheless, previous studies have suggested that both human epidermal keratinocytes and dermal fibroblasts are capable of synthesizing type VII collagen (9). In this study, we have examined type VII collagen gene expression more precisely at the mRNA level. This approach has been facilitated by the isolation of type VII collagen cDNA clones (11). The clone used in this study (K-131) encompasses both a noncollagenous and a collagenous domain, and it has been demonstrated that this cDNA clearly corresponds to type VII collagen sequences (11).

Northern hybridizations with the cDNA K-131 recognized an mRNA transcript that was estimated to be ~ 9 kb in size. This estimation was based on hybridizations of the same Northern filters containing RNA from epidermal keratinocytes with a bullous pemphigoid antigen-1 (230-kD protein) cDNA that recognized a transcript of ~ 9.0 kb (21), and subsequent

hybridizations of filters containing RNA from skin fibroblasts that revealed the presence of proα1(I) collagen mRNAs 5.8 and 4.8 kb, respectively (27). The ~ 9-kb type VII collagen mRNA would maximally encode a polypeptide of ~ 2,900 amino acids, allowing for the presence of small 5′ and 3′ untranslated regions in the mRNA. Such a polypeptide would have a molecular mass of ~ 290 kD (with an average molecular mass of 100 D for each amino acid). This size is slightly smaller than the estimated molecular mass of individual proα1(VII) chains, 300–350 kD (3). The difference in size could be explained by posttranslational modification of the polypeptides, including *N*-glycosylation of the noncollagenous portion of the protein (30), as well as the synthesis of hydroxyproline and hydroxylysine, with subsequent *O*-glycosylation of hydroxylysyl residues, in the collagenous domain (31). Thus, the estimated size of type VII collagen mRNAs, ~ 9 kb, is compatible with the estimated size of proα1(VII) polypeptides.

Previous studies have suggested that type VII collagen is synthesized both by epidermal keratinocytes and dermal fibroblasts (9, 29), but the relative levels of gene expression and the potential contribution of these cell types to the synthesis of anchoring fibrils have not been elucidated. Our results indicate that total RNA isolated from epidermal keratinocytes contains significantly higher levels of type VII collagen mRNAs than RNA isolated from dermal fibroblasts, when examined in parallel hybridizations (see Fig. 2). This impression was corroborated by parallel immunofluorescence staining of these cell cultures (Fig. 4). Interestingly, the apparent intensity of the immunofluorescence signal was enhanced in co-cultures containing a mixture of keratinocytes and fibroblasts (Fig. 4). This conclusion supports recent observations by König and Bruckner-Tuderman (29) demonstrating that primary skin explants under culture conditions supporting growth of both cell types, or mixed co-cultures of purified fibroblasts and keratinocytes, exhibited clearly enhanced synthesis of type VII collagen. These authors concluded that epithelial-mesenchymal interactions are necessary for efficient synthesis of type VII collagen and biogenesis of the anchoring fibrils (29). The mechanisms leading to enhanced type VII collagen synthesis in these co-cultures are not clear. However, in situ hybridizations of cultured cells suggested that the amount of type VII collagen mRNA was enhanced in keratinocytes that were in direct cell–cell contact. This observation would suggest that cell–cell interactions could lead to increased synthesis and deposition of type VII collagen by the same cells.

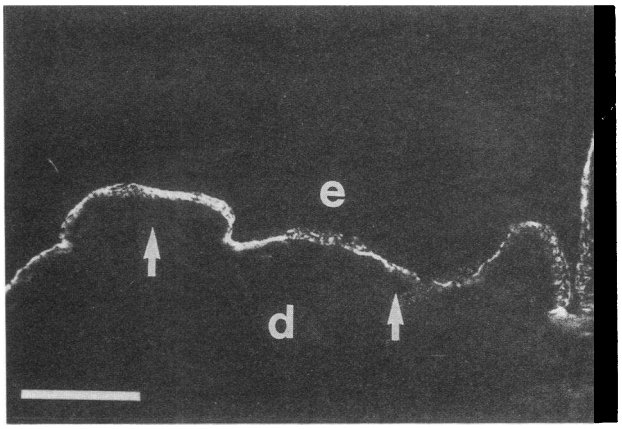

Figure 5. Demonstration of type VII collagen gene expression in human fetal skin by indirect immunofluorescence. Note that staining of a skin specimen from a 19-wk fetus reveals the presence of antigenic epitopes at the dermal–epidermal basement membrane zone (*arrows*) between the epidermis (*e*) and the dermis (*d*). Original magnification: bar, 100 μm.

Previous studies have also suggested that spontaneously transformed epithelial cells, such as WISH cells (a human amniotic epithelial cell line) or KB cells (a human oral epidermoid carcinoma cell line), synthesize type VII collagen (28). Northern analyses demonstrated that these cell lines expressed the type VII collagen gene in culture. However, the level of expression by WISH cells was low when compared with KB cells in the same hybridization experiments (see Fig. 2 *C*). These conclusions were also supported by immunofluorescence data, which demonstrated a significantly higher signal in KB cell cultures in comparison to WISH cells, again studied in parallel. Thus, KB cells provide a convenient cell culture system to study modulation of type VII collagen gene expression. The level of type VII collagen mRNAs in virally transformed human keratinocytes, RHEK and HPK cell lines, was relatively low. This observation is consistent with previous reports indicating that viral transformation of a variety of cells leads to diminished expression of other collagen genes (see 32, 33).

Immunostaining of skin from a 19-wk fetus demonstrated the presence of type VII collagen epitopes in a linear fashion along the dermal–epidermal basement membrane zone (Fig. 5). Examination of the dermis in the same skin preparation did not reveal a significant level of immunofluorescence. These observations, together with in vitro data suggest that the basal keratinocytes are primarily responsible for the synthesis of type VII collagen in developing fetal skin. Since basal keratinocytes are located on the epidermal side of the developing basement membrane, this observation raises the question of the mechanisms by which the newly synthesized type VII collagen molecules are transported across the basement membrane zone and eventually deposited in the anchoring fibrils. It is conceivable that during early fetal development, the barrier function of the basement membrane is relatively incomplete and the type VII collagen molecules can easily traverse it (34). However, in ma-

Figure 4. Detection of type VII collagen epitopes in cultured cells by indirect immunofluorescence with a monoclonal antibody L_3D. Note the presence of clearly detectable staining in a granular pattern in the cytoplasm of epidermal keratinocytes (*A*), while the signal is low in dermal fibroblasts (*B*). Coculture of keratinocytes (*) and fibroblasts (*arrow*), which were plated in a 10:1 ratio (~ 3×10^3 cells per cm^2) and examined at 48 h of incubation, appears to accentuate the immunofluorescence signal in both cell types (*C*) when examined in parallel with cultures shown in *A* and *B*. The extremely bright areas in *C* reflect the high level of type VII collagen epitopes in clustered keratinocytes (*), when compared with fibroblasts in the same culture or when compared to individual keratinocytes in *A* examined in parallel. Human amniotic epithelial WISH cells reveal a relatively low level of immunofluorescence signal (*D*), while KB cells, a human oral epidermoid carcinoma cell line, reveal a higher level of type VII collagen epitopes (*E*). The cultures shown in *A–C* were examined in parallel in one experiment, while those shown in *D* and *E* were stained in parallel in another experiment. The photographic exposure time was the same for all cultures, and the prints were reproduced under identical conditions. Original magnification is the same in all pictures: bar, 50 μm.

ture skin during the postnatal period, an intact basement membrane may not allow a significant amount of type VII collagen to be transported to the dermal side. This may explain the observations that the synthesis of anchoring fibrils by keratinocytes placed upon dermis in patients with cutaneous burns is extremely slow and incomplete (35). It is also conceivable that in these situations, synthesis and transport of type VII collagen molecules into the dermal side of the basement membrane may occur, but other events necessary for assembly of functional anchoring fibrils are delayed.

In summary, in this study we have utilized a recently cloned human cDNA for the detection of type VII collagen gene expression in cultured human cells. The results demonstrate expression of the type VII collagen gene by a variety of human cells in culture, including keratinocytes and fibroblasts, however epidermal keratinocytes may be the primary source of type VII collagen in anchoring fibrils. Utilization of these cDNA probes will be of help in further elucidation of the regulatory mechanisms governing the synthesis and secretion of type VII collagen and its assembly into functional anchoring fibrils.

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References

1. Adams, S. L. 1989. Collagen gene expression. *Am. J. Respir. Cell Mol. Biol.* 1:161–168.

2. Vuorio, E., and B. de Crombrugghe. 1990. The family of collagen genes. *Annu. Rev. Biochem.* 59:837–872.

3. Burgeson, R. E., G. P. Lunstrum, B. Rokosova, C. S. Rimberg, L. M. Rosenbaum, and D. R. Keene. 1990. The structure and function of type VII collagen. *Ann. NY Acad. Sci.* 580:32–43.

4. Keene, D. R., L. Y. Sakai, G. P. Lunstrum, N. P. Morris, and R. E. Burgeson. 1987. The type VII collagen forms an extended network of anchoring fibrils. *J. Cell Biol.* 104:611–621.

5. Lunstrum, G. P., L. Y. Sakai, D. R. Keene, N. P. Morris, and R. E. Burgeson. 1986. Large complex of globular domains of type VII procollagen contribute to the structure of anchoring fibrils. *J. Biol. Chem.* 261:9042–9048.

6. Morris, N. P., D. R. Keene, R. W. Glanville, H. Bentz, and R. E. Burgeson. 1986. The tissue form of type VII collagen is an antiparallel dimer. *J. Biol. Chem.* 261:5638–5644.

7. Ghadially, F. N. 1988. Ultrastructural Pathology of the Cell and Matrix. 3rd edition. Volume 2. Butterworths, London. 1228–1230.

8. Tidman, M. J., and R. A. J. Eady. 1985. Evaluation of anchoring fibrils and other components of the dermal–epidermal junction in dystrophic epidermolysis bullosa by a quantitative ultrastructural technique. *J. Invest. Dermatol.* 84:374–377.

9. Stanley, J. R., N. Rubenstein, and V. Klaus-Kovtun. 1985. Epidermolysis bullosa acquisita antigen is synthesized by both human keratinocytes and human dermal fibroblasts. *J. Invest. Dermatol.* 85:542–545.

10. Bruckner-Tuderman, L., U. W. Schnyder, K. H. Winterhalter, and P. Bruckner. 1987. Tissue form of type VII collagen from human skin and dermal fibroblasts in culture. *Eur. J. Biochem.* 165:607–611.

11. Parente, M. G., L. C. Chung, J. Ryynänen, D. T. Woodley, K. C. Wynn, E. A. Bauer, M.-G. Mattei, M.-L. Chu, and J. Uitto. 1991. Human type VII collagen: cDNA cloning and chromosomal mapping of the gene. *Proc. Natl. Acad. Sci. USA.* 88:6931–6935.

12. Olsen, D. R., N. J. Hickok, and J. Uitto. 1990. Suppression of ornithine decarboxylase gene expression by retinoids in cultured human keratinocytes. *J. Invest. Dermatol.* 94:33–36.

13. Booth, B. A., K. L. Polak, and J. Uitto. 1980. Collagen biosynthesis by human skin fibroblasts. I. Optimization of the culture conditions for synthesis of type I and type III procollagens. *Biochim. Biophys. Acta.* 607:145–160.

14. Hayflick, L. 1961. Propagation in fluid medium of human epidermoid carcinoma, strain KB. *Proc. Soc. Exp. Biol. Med.* 89:362–364.

15. Fogh, J., and R. Lung. 1957. Continuous cultivation of epithelial cell strain (FL) from human amniotic membrane. *Proc. Soc. Exp. Biol.* 94:532–537.

16. Dürst, M., D. Gallahan, G. Jay, and J. S. Rhim. 1989. Glucocorticoid-enhanced neoplastic transformation of human keratinocytes by human papillomavirus type 16 and an activated ras oncogene. *Virology.* 173:767–771.

17. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

18. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5205.

19. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 10.13–10.17.

20. Chu, M.-L., J. C. Myers, M. P. Bernard, J.-F. Ding, and F. Ramirez. 1982. Cloning and characterization of five overlapping cDNAs specific for the human proα1(I) collagen chain. *Nucleic Acids Res.* 10:5925–5934.

21. Sawamura, D., K.-H. Li, K. Nomura, Y. Sugita, A. M. Christiano, and J. Uitto. 1991. Bullous pemphigoid antigen: cDNA cloning, cellular expression, and evidence for polymorphism of the human gene. *J. Invest. Dermatol.* 96:908–915.

22. Fort, P., L. Marty, M. Piechaczyk, S. El Sabrouty, C. Danz, P. Jeanteur, and J. M. Blanchard. 1985. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucleic Acids Res.* 13:1431–1442.

23. Harper, M. E., L. M. Marselle, R. C. Gallo, and F. Wong-Staal. 1986. Detection of lymphocytes expressing human T-lymphotrophic virus type III in lymph nodes and peripherial blood from infected individuals by in situ hybridization. *Proc. Natl. Acad. Sci. USA.* 83:772–776.

24. Scharffetter, K., B. Lankat-Buttgereit, and T. Krieg. 1988. Localization of collagen mRNA in normal and scleroderma skin by in situ hybridization. *Eur. J. Clin. Invest.* 18:9–17.

25. Paller, A. S., L. L. Queen, D. T. Woodley, E. J. O'Keefe, W. R. Gammon, and R. A. Briggaman. 1985. A mouse monoclonal antibody against a newly discovered basement membrane component, the epidermolysis bullosa acquisita antigen. *J. Invest. Dermatol.* 82:215–217.

26. Seltzer, J. L., A. Z. Eisen, E. A. Bauer, N. P. Morris, R. W. Glanville, and R. E. Burgeson. 1989. Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin. *J. Biol. Chem.* 264:3822–3826.

27. Ohta, A., and J. Uitto. 1987. Procollagen gene expression by scleroderma fibroblasts in culture: inhibition of collagen production and reduction of proα1(I) and proα1(III) collagen messenger RNA steady-state levels by retinoids. *Arthritis Rheum.* 30:404–411.

28. Sakai, L. Y., D. R. Keene, N. P. Morris, and R. E. Burgeson. 1986. Type VII collagen is a major structural component of anchoring fibrils. *J. Cell Biol.* 103:1577–1586.

29. König, A., and L. Bruckner-Tuderman. 1991. Epithelial-mesenchymal interactions enhance expression of collagen VII in vitro. *J. Invest. Dermatol.* 96:803–808.

30. Hart, G. W., K. Brew, G. A. Grant, R. A. Bradshaw, and W. J. Lennarz. 1979. Primary structural requirements for the enzymatic formation of the N-glycosidic bond in glycoproteins. *J. Biol. Chem.* 254:9747–9753.

31. Prockop, D. J., R. A. Berg, K. I. Kivirikko, and J. Uitto. 1976. Intracellular steps in the biosynthesis of collagen. *In* Biochemistry of Collagen. G. N. Ramachandran, and A. J. Reddi, editors. Plenum Publishing Corp., New York. 163–273.

32. Adams, S. L., M. Pacifici, R. J. Focht, E. S. Allebach, and D. Boettiger. 1985. Collagen synthesis in virus-transformed cells. *Ann. NY Acad. Sci.* 460:202–213.

33. Chan, L.-M., C. Hatier, G. Parry, Z. Werb, and M. J. Bissell. 1987. Collagen fibronectin interactions in normal and Rous sarcoma virus-transformed avian tendon cells: possible mechanisms for increased extracellular matrix turnover after transformation in vitro. *In Vitro Cell Dev. Biol.* 23:308–314.

34. Smith, L. T., L. Y. Sakai, R. E. Burgeson, and K. A. Holbrook. 1988. Ontogeny of structural components at the dermal–epidermal junction in human embryonic and fetal skin: the appearance of anchoring fibrils and type VII collagen. *J. Invest. Dermatol.* 90:480–485.

35. Woodley, D. T., H. D. Peterson, S. R. Herzog, G. P. Stricklin, R. E. Burgeson, R. A. Briggaman, D. J. Cronce, and E. J. O'Keefe. 1988. Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstruction of anchoring fibrils. *JAMA (J. Am. Med. Assoc.).* 259:2566–2571.