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Sequence and Expression of a Type II Keratin, K5, in Human Epidermal Cells

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We report here the cDNA and amino acid sequences of a human 58-kilodalton type II keratin, K5, which is coexpressed with a 50-kilodalton type I keratin partner, K14, in stratified squamous epithelia. Using a probe specific for the 3'-noncoding portion of this K5 cDNA, we demonstrated the existence of a single human gene encoding this sequence. Using Northern (RNA) blot analysis and in situ hybridization with cRNA probes for both K5 and K14, we examined the expression of these mRNAs in the epidermis and in cultured epidermal cells. Our results indicate that the mRNAs for K5 and K14 are coordinately expressed and abundant in the basal layer of the epidermis. As cells undergo a commitment to terminally differentiate, the expression of both mRNAs seems to be downregulated.

Keratin filaments constitute a group of 8-nm fibers that form an integral and unique part of the cytoskeleton of almost all higher eucaryotic epithelial cells (33, 44). In humans, there are more than 20 different keratins (40 to 67 kilodaltons [kDa]) encoded by a large multigene family (10). Keratins can be subdivided into two distinct sequence classes, type I and type II (10, 19, 25). Type I keratins are generally small (40 to 56.5 kDa) and relatively acidic (pKi, 4.5 to 5.5), while type II keratins are larger (53 to 67 kDa) and more basic (pKi, 6.5 to 7.5) (14, 25, 33, 40, 44). Type I and type II keratins are frequently expressed as specific pairs, and at least one pair of keratins is always expressed in any epithelial cell (8). Changes in differentiation (13, 48, 50) and development (7, 35) in epithelial cells often coincide with alterations in keratin synthesis, suggesting that the expression of keratins might be finely tailored to suit the particular and varied structural requirements of each epithelial cell.

In the epidermis, keratin synthesis is especially abundant, leaving the fully differentiated squames with 85% of their protein as keratin. As judged with keratin extracts from epidermal sections cut parallel to the skin surface, the pattern of keratins undergoes change as an epidermal cell undergoes a commitment to terminally differentiate (13). In the basal layer, two keratins are expressed: a type I 50-kDa keratin, K14, and a type II partner 58-kDa keratin, K5 (36). As a differentiating epidermal cell migrates outward toward the skin surface, it synthesizes new keratins of both the type I class (K10 and K11) and the type II class (K1 and K2) (13, 24, 35, 44, 50). Immunofluorescence studies have shown that the induction of the large keratins appears to take place in the first suprabasal layer (27, 35, 48). Analysis of keratins from tissue sectioning has indicated that the K5-K14 pair may also be present in the suprabasal layers (13, 50). However, owing to (i) the undulating nature of the epidermis and (ii) the masking of some critical antigenic determinants of the keratins in the suprabasal layers (50), it has been difficult to assess to what extent the expression of the basal keratins continues during terminal differentiation and whether this expression is a consequence of a stable preexisting protein or new protein synthesis.

To elucidate the molecular mechanisms underlying the differential expression of keratin pairs in the epidermis, we previously isolated and characterized a cDNA clone (18) and a gene (29, 30) for K14 (50 kDa). We now report the isolation and characterization of a cDNA encoding its partner keratin, K5. We examine its complexity in the human genome and explore the coordinate expression of K5 and K14 in the epidermis and in cultured epidermal cells.

Isolation and identification of a cDNA encoding human keratin K5. Previously, a pBR322 cDNA library was prepared from cultured human epidermal cell mRNAs, which include keratin mRNAs for the type II keratins K5 and K6 and the type I keratins K13, K14, K16, and K17 (10). Using the procedure of Grunstein and Hogness (17), we screened this library for clones which hybridized with ^{32}P-labeled cDNA prepared from a 1,415-base-pair (bp) *Taq*I-*Xmn*I cDNA fragment encoding a portion of the human type II keratin K6 (10, 47). The parent of this subclone, pKA-1, was previously shown to hybridize at a high stringency with K6 mRNAs and at a reduced stringency with both K6 and K5 mRNAs (25). Therefore, after hybridization with the radiolabeled K6 probe, filters were washed at two temperatures based on the calculated melting temperature for a hybrid formed between the cDNA probe and a putative cloned K6 cDNA 1,000 bp long (2): high stringency was set at 5 to 10°C below the calculated melting temperature, and low stringency was set at 15 to 20°C below the calculated melting temperature. One plasmid, pKA-62, which hybridized after the low-stringency (65°C) wash but not after the high-stringency (75°C) wash, was selected for further study.

To identify the keratin encoded by this clone, we denatured and immobilized plasmid pKA-62 on a nitrocellulose filter and used it in a positive hybridization and translation assay (38). After exposure of total epidermal cell poly(A)$^+$ RNAs to cloned DNAs from pKA-62 and pKA-1 (K6 cDNA), specifically hybridizing mRNAs were eluted at either 65 or 85°C, translated in vitro, and analyzed by polyacrylamide gel electrophoresis and fluorography. The translation products of the plasmid-selected mRNAs are

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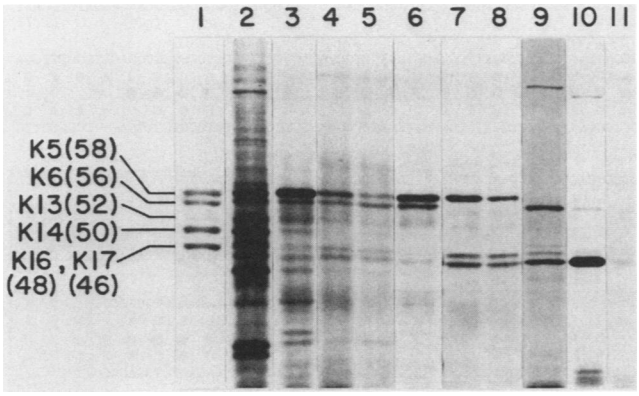

FIG. 1. Translation of cultured human epidermal cell mRNAs eluted from filter-immobilized keratin cDNAs. Linearized whole plasmid cDNAs (20 μg) were denatured and immobilized on nitrocellulose filters, and the filters were hybridized with 1 μg of poly(A)$^+$ RNAs isolated from human epidermal cells. After being washed at 50°C to remove unhybridized RNAs, specifically bound mRNAs were sequentially eluted with H_2O first at 65°C and then at 85°C. Eluted RNAs were translated in a reticulocyte lysate system in the presence of [^{35}S]methionine, and the translation products were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The translation products were visualized by fluorography and autoradiography. Radiolabeled proteins were from a [^{35}S]methionine-labeled keratin extract from cultured human epidermal cells (lane 1); translated products from cultured human epidermal poly(A)$^+$ RNA (lane 2); and translated products with no mRNA added (artifacts of the reticulocyte translation) (lane 11). Lanes 3 to 10 represent translation products from mRNAs eluted at 65°C (lanes 3, 5, and 7) or 85°C (lanes 4, 6, 8, 9, and 10). The filters contained the following DNAs: pKA-1 (lanes 3 and 4); pKA-62 (lanes 5 and 6); a specific 3′-noncoding segment of KA-62 (a 190-bp *Nco*I-*Hae*III fragment beginning 44 nucleotides 3′ from the TAA stop codon and extending to the 3′ end of the clone) (lanes 7 and 8); a specific 3′-noncoding segment of pKA-1, the K6a cDNA (47) (lane 9); and a specific 3′-noncoding segment of the K6b cDNA (47) (lane 10). Molecular masses are in kilodaltons at left. Keratins are identified according to the numbering system of Moll et al. (33).

shown in Fig. 1. Both plasmid DNAs hybridized with mRNAs encoding K5 and K6. However, at 65°C, more than 80% of the K5 mRNAs and only 50% of the K6 mRNAs were eluted from pKA-1 (Fig. 1, lane 3), whereas 50% of the K6 mRNAs and only 20% of the K5 mRNAs were eluted from pKA-62 (Fig. 1, lane 5). At 85°C, all of the remaining K5 and K6 mRNAs were eluted (Fig. 1, lanes 4 and 6). On the basis of these data, we tentatively identified pKA-62 as a clone encoding the human basal epidermal keratin K5.

After sequencing the keratin cDNA (see below), we repeated our positive hybridization and translation analyses, this time using a 3′-noncoding-portion subclone of KA-62. In contrast to the results obtained with the entire KA-62 cDNA (Fig. 1, lanes 5 and 6), the 3′-noncoding cDNA selected only the mRNA encoding a 58-kDa keratin, even under reduced-stringency conditions (Fig. 1, lanes 7 and 8). Similarly, 3′-noncoding cDNAs for K6a (Fig. 1, lane 9) and K6b (Fig. 1, lane 10) were specific and selected only the mRNAs encoding a 56-kDa keratin (47). These results provide verification that the cDNA contained in KA-62 encodes the human keratin K5. Southern blot analysis (41; data not shown) with the 3′-noncoding probe indicated that there is a single copy of the gene encoding the KA-62 mRNA in the genome and that the probe is selective for this K5 gene and its transcript.

Sequencing strategy, nucleotide sequence, and predicted secondary structure of the type II keratin cDNA contained in clone pKA-62. The strategy used in determining the nucleotide sequence of the KA-62 cDNA insert is shown in Fig. 2. The complete sequence of the KA-62 cDNA insert is shown in Fig. 3 along with its optimal alignment to the highly similar coding sequence for the gene encoding human K6b (46). The exact size of the KA-62 insert is 1,869 nucleotides. The open reading frame shown in Fig. 3 extends for 1,527 nucleotides and ends with the translation termination codon TAA. A 3′-untranslated sequence of 342 nucleotide residues extends past the stop codon. No AATAAA polyadenylation signal sequence (9) was found within this sequence, nor was there a poly(A) stretch prior to the 21 cytosine residues that mark the complementary nucleotides added to the *Pst*I site of pBR322 in the course of cloning. Thus, it is likely that the 3′-noncoding sequences of KA-62 represent only part of the 3′-noncoding portion of the K5 mRNA.

To provide evidence that the open reading frame is indeed the correct one for K5, we isolated the 58-kDa K5 protein from cultured human epidermal cells and determined its amino acid composition. The amino acid analysis was in good agreement with the amino acid composition predicted on the basis of the cDNA sequence (Table 1). However, the percentages of glycine, serine, and phenylalanine residues were higher when determined from the protein analysis than when determined from the predicted sequence. Since other epidermal type II keratins have been shown to have amino-terminal sequences which are rich in these residues, we expect that the missing amino-terminal portion of the cDNA sequence codes for a protein segment that is similar to the amino-terminal portion of human K6 (46) or K1 (21). With this assumption, the amino acid composition of the predicted portion of the putative K5 sequence corresponds very well to the actual amino acid analysis.

The predicted amino acid sequence of the putative K5 keratin encoded by the KA-62 cDNA is similar to the sequence of the K6b keratin (Fig. 3) (46). Only 88 amino acid residues are different, and many of the changes are conservative ones (e.g., Asp → Glu or Ile → Val). The greatest identity (88.9%) resides in the central 316 amino acid residues of the K5 and K6b polypeptides (corresponding to residues 159 to 474 of the K6b chain [Fig. 3 and Tables 2 and 3]). In this sequence are four segments (marked by the shaded areas in Fig. 3) which are predicted to be largely α-helical. Throughout these segments is a periodicity (31, 32)

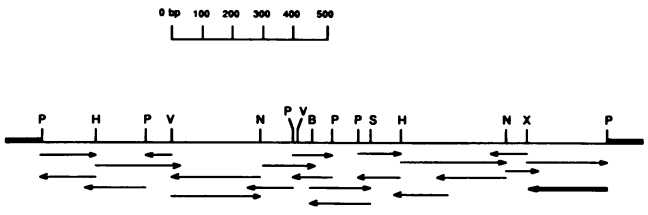

FIG. 2. DNA sequencing strategy for the human type II keratin KA-62 cDNA. A restriction map of the KA-62 insert (thin line) flanked by pBR322 sequences (thick lines) is shown to scale. The direction of the mRNA strand from left to right is 5′ to 3′, and the positions of all recognition sites for each enzyme are indicated as follows: P, *Pst*I; H, *Hin*cII; N, *Nco*I; V, *Pvu*II; S, *Sac*I; and X, *Xba*I. Thin arrows designate the direction and extent of the DNA sequence determined for each fragment. All sequencing was done by the dideoxy nucleotide method of Sanger et al. (39). The thick arrow represents sequence information obtained with a synthetic oligonucleotide primer prepared for a sequence near the 3′ end of KA-62: 5′-CACATTCTGGAGGTAGT-3′

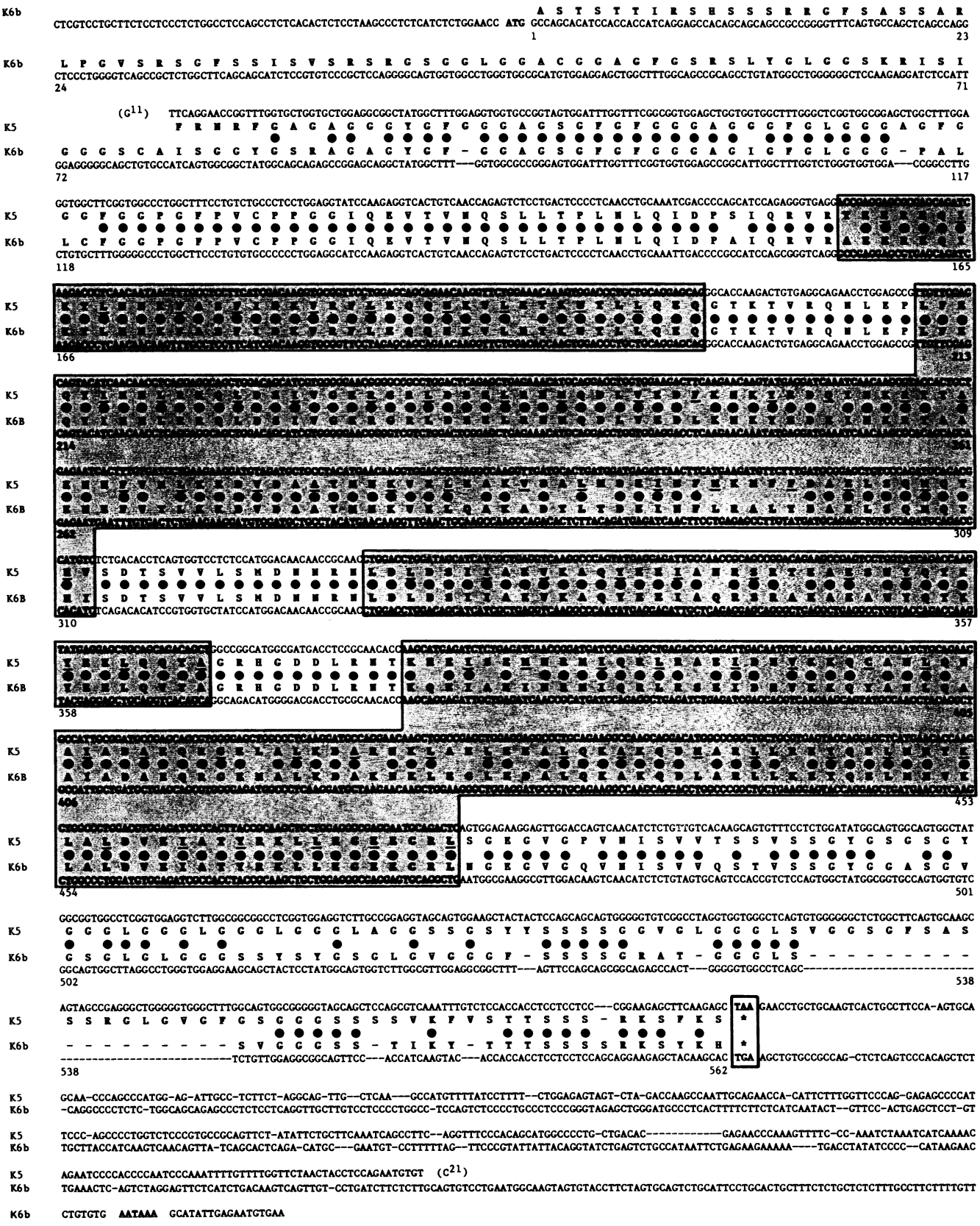

FIG. 3. Comparison of the complete nucleotide and predicted amino acid sequences of the human type II keratin cDNA KA-62 with those of K6b cDNA. The sequence is shown in the 5′-to-3′ direction of the mRNA strand. The amino acid and nucleotide sequences of the K5 cDNA (this paper) and the K6b cDNA (46) are aligned for optimal homology. The numbers mark the positions of the amino acid residues of the K6b keratin as described previously (46). The solid circles indicate positions of identity in the amino acid sequences. In the coding region, the sequences share 83% homology at the amino acid level and 82% homology at the nucleotide sequence level. The shaded areas mark the boundaries of the predicted α-helical domains in the two sequences (3, 4, 19). Throughout these domains are the heptad repeats of hydrophobic amino acid residues, which identify the portions of the polypeptides that are involved in coiled-coil interactions with a second keratin (31, 32). These hydrophobic residues are underlined. The translation stop codon TAA is encased in a box.

TABLE 1. Comparison of the amino acid compositions of the 58-kDa human epidermal keratin K5 and the keratin encoded by KA-62

Amino acid	% as determined by:	
	Amino acid analysis[a]	cDNA sequence[b]
Ala	6.4	6.3
Arg	5.2	5.7
Asn		5.3
Asp		4.5
Asn + Asp	9.4	9.8
Cys	ND[c]	0.6
Gln		5.3
Glu		7.9
Gln + Glu	13.0	13.2
Gly	16.9	15.0
His	0.9	0.8
Ile	3.6	3.5
Leu	9.6	9.4
Lys	5.1	5.5
Met	1.7	2.4
Phe	5.0	4.3
Pro	1.6	1.6
Ser	10.8	8.7
Thr	4.3	4.5
Trp		0.4
Tyr	2.8	2.6
Val	5.0	5.7

[a] Keratins were isolated from cultured human epidermal cells, and individual polypeptides were separated and purified by polyacrylamide gel electrophoresis and electroelution (11). Glycine was used in the gels and buffers for one portion, and the substitution of molar equivalents of alanine for glycine was used for another portion. Electroeluted samples were dialyzed against 10 mM Tris hydrochloride (pH 7.6) prior to precipitation with trichloroacetic acid. Aliquots of 20 μg of the purified 58-kDa keratin were hydrolyzed in 6 M HCl at 108°C for 36 h in evacuated sealed tubes. After being vacuum dried, samples were applied to a Dionex D-501 amino acid analyzer.

[b] Calculated from the predicted protein sequence in Fig. 3. A total of 508 residues were sequenced.

[c] ND, Not determined.

in which most of the first and fourth residues of every seven amino acids in the sequence are hydrophobic (indicated as underlined amino acid residues in Fig. 3). The high degree of sequence identity, the predicted α-helicity, and the heptad repeats of hydrophobic residues throughout the central 316 amino acid residues are characteristic of all type II keratins which have been sequenced thus far (6, 15, 19, 20, 21, 26, 28, 42, 46). Within this domain, type I keratins are similar in predicted secondary structure and heptad repeats, but they share only 25 to 35% homology with the type II keratins (18, 23, 29, 43).

TABLE 2. Amino acid identities of K5 and K6 proteins from different species

Keratins compared	% Amino acid identity			
	Head	α-Helical domain	Tail	Total protein
Human K5[a] vs human K6b[b]	82.7	88.9	62.1	82.9
Human K5 vs mouse 60-kDa keratin[c]	70.7	81.6	63.2	76.5
Human K5 vs bovine keratin III[d]		92.1	91.6	91.8
Human K6b vs mouse 60-kDa keratin	75.0	89.9	78.1	84.0

[a] This paper.
[b] Tyner et al. (46).
[c] Steinert et al. (42).
[d] Jorcano et al. (22).

TABLE 3. Nucleotide identities of K5 and K6 mRNAs from different species

Keratins compared	% Nucleotide identity			
	Sequence coding for head	Sequence coding for α-helical domain	Sequence coding for tail	Total mRNA (coding)
Human K5[a] vs human K6b[b]	79.4	88.0	61.7	81.8
Human K5 vs mouse 60-kDa keratin[c]	70.3	82.5	62.4	76.8
Human K5 vs bovine keratin III[d]		91.5	88.5	89.6
Human K6b vs mouse 60-kDa keratin	78.4	88.3	83.9	84.9

[a] This paper.
[b] Tyner et al. (46).
[c] Steinert et al. (42).
[d] Jorcano et al. (22).

The most divergent region between K5 and K6b is the nonhelical carboxy-terminal end (corresponding to residues 475 to 562 of K6b), in which there is only 62.1% identity (Table 2). In addition, at a position equivalent to residue 538 of K6b, there is a string of 18 amino acid residues in K5 which does not have a counterpart in K6b. Interestingly, a putative mouse K5 keratin reportedly homologous to the human 58-kDa K5 keratin (42) is more similar to human K6b than to human K5 in that it is missing these 18 amino acid residues. Moreover, throughout the carboxy-terminal domain, the putative mouse K5 has a much stronger sequence identity to human K6b than to human K5 (Tables 2 and 3). In contrast, the part of bovine keratin III beginning at 54 residues prior to the highly conserved L L E G E sequence and extending to the carboxy terminus (22) shares 92% identity with human K5. These data indicate that the bovine keratin III sequence is closer in evolutionary origin to the human sequence contained in pKA-62 than is the mouse 60-kDa sequence reported by Steinert et al. (42). On the basis of these data, the mouse 60-kDa sequence most likely encodes a K6 keratin rather than a K5 keratin.

Expression of the human K5 mRNA in the epidermis and in cultured epidermal cells. To examine the expression of K5 mRNA in the epidermis and in cultured epidermal cells, we isolated poly(A)$^+$ mRNAs from human skin and from cultured epidermal cells. Northern (RNA) blot analysis (45) of these mRNAs indicated that the radiolabeled 3'-noncoding probe for K5 hybridized with a single mRNA band (2.1 kilobases) expressed in both cultured human epidermal cells (Fig. 4, lane 2) and human epidermis (Fig. 4, lane 3). This mRNA was not detected in the human breast carcinoma line MCF-7 (Fig. 4, lane 1), which expresses only K8 of the type II family and K18 and K19 of the type I family (33). The size of the probe-selected mRNA was consistent with previous estimates of K5 mRNA based upon electrophoretic mobilities of the mRNA on denaturing agarose gels (12). On this basis, we conclude that the cDNA contained in KA-62 represents approximately 90% of the complete mRNA encoding human K5.

To examine the localization of K5 and K14 mRNAs in human skin, we prepared ^{35}S-labeled cRNA probes corresponding to the coding and noncoding portions of K5 (KA-62) and K14 (KB-2) (18, 47). These probes were hybridized

in situ to cross-sections of human skin (5, 47). Autoradiography revealed that both K5 and K14 mRNAs hybridized predominantly to the basal epidermal layer of human skin, although some hybridization to the suprabasal layers was seen (Fig. 5A and B). Silver grains were concentrated most heavily in the cytoplasmic regions of the epidermal cells. Few grains were seen over the dermis, and only background-level labeling was found in the stratum corneum.

The localizations observed with the 3′-noncoding segments of K5 and K14 mRNAs were similar to those seen with the total probes. Moreover, hybridization with the K5 probe was markedly different from that with a K6 probe, in which the mRNA was evenly distributed throughout the metabolically active layers of the epidermis even though the K6 protein is not normally expressed in this tissue (47). Thus, the sequence differences between K5 and K6 mRNAs seemed to be sufficient to prevent cross-hybridization, even when the complete cRNAs were used as probes.

To investigate the localization of K5 and K14 mRNAs in cultured cell colonies, we carefully dislodged the stratified cells from the culture dish with the enzyme dispase (16) and fixed and embedded the "tissue" as we did for human skin (5, 47). In situ hybridizations with the K5 and K14 probes revealed a distribution similar to that observed for skin (Fig. 5C and D). Thus, the two epidermal cell mRNAs seemed to be coordinately regulated in both skin and cultures, and their expression was especially abundant in the basal epidermal layer.

Conclusions. In this paper, we have described the isolation and characterization of a cDNA encoding the human keratin K5, a protein which, with its partner keratin K14, is expressed in all stratified squamous epithelial cells of the body, including the epidermis. This pair of keratins is highly similar in sequence and in structure to another pair of keratins, K6 (19, 46) and K16 (37), which are only expressed in the epidermis under conditions of hyperproliferation (49; E. Birgitte Lane, personal communication). Although our KA-62 clone does not contain the sequences coding for the N-terminal portion of K5, it is likely that most of the size variation between K5 and K6 resides in the carboxy terminus of the polypeptides: K5 is 21 amino acid residues longer in this region, and its carboxy-terminal sequence is 1,482 daltons larger than the corresponding region of K6b. A comparison of the sequence data available for K5 reveals that the entire polypeptide encoded by KA-62 is 2,034 daltons larger than the corresponding region of K6b. Since electrophoretic analysis reveals an apparent molecular mass for K5 which is only 2,000 daltons greater than that for K6, it is unlikely that substantial differences will exist in the amino-terminal lengths of the two polypeptide chains.

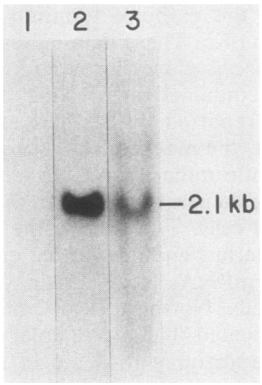

FIG. 4. Northern blot analysis of different epithelial cell RNAs hybridized with a specific K5 keratin probe. Poly(A)$^+$ RNAs were isolated from MCF-7 human breast adenocarcinoma cells (lane 1) and cultured human epidermal cells (lane 2) as described by Berk and Sharp (1). Total RNA was isolated from human foreskin tissue (lane 3). MCF-7 mRNA (0.5 μg), 0.5 μg of cultured epidermal cell mRNA, and 27 μg of total foreskin RNA were resolved by formaldehyde-agarose gel electrophoresis. After being transferred to nitrocellulose paper by blotting (45), the RNAs were hybridized with a ^{32}P-labeled cRNA probe transcribed from a 3′-noncoding probe complementary to and specific for K5 mRNA. Hybridization and washes were carried out as described by Thomas (45). Lanes 1 and 3 were exposed to X-ray film for 3 days, and lane 2 was exposed for 12 h. The size of the detected RNA band was determined by yeast and mammalian rRNA markers and is shown at the right in kilobases.

We do not yet know precisely how many K5 genes exist in the human genome. Although we used a 3′-noncoding-portion subclone and identified only a single gene with this probe, this result does not exclude the possibility that multiple K5 genes with different 3′-noncoding segments exist. In fact, although a 3′-noncoding probe for K14 selects only a single genomic fragment (29), a probe corresponding to intron I of the K14 gene hybridizes to three different genomic fragments (M. Rosenberg, A. RayChaudhury, T. B. Shows, M. LeBeau, and E. Fuchs, Mol. Cell. Biol., in press). Similarly, there are two distinct genes encoding K6: 125 bp 3′ downstream from their TGA stop codons, the noncoding portions of the two sequences diverge (46, 47). Since probes encompassing the coding segments of highly similar genes cross-hybridize extensively, a precise determination of the number of genes encoding a specific keratin must await the isolation and characterization of the genomic clones. However, in all of the cases examined thus far, only one of the multiple genes encoding a single keratin seems to have been expressed in abundance (47; Rosenberg et al., in press).

Because we found the 3′-noncoding probe for KA-62 to be unique in the human genome, Northern blot analysis with this probe could be used to demonstrate unequivocally that the gene encoding this K5 sequence is expressed efficiently both in cultured epidermal cells and in the epidermis. Once a thorough study of mRNAs from other stratified squamous epithelia has been made with the KA-62 probe, we expect to find that this sequence is also expressed in at least some, if not all, of the other tissues in which K5 is typically present. Interestingly, the localizations of K5 and K14 mRNAs in skin were highly similar: both of these mRNAs were expressed predominantly in the basal epidermal layer, but they clearly persisted in the suprabasal layers as well. In cultured epidermal cells, the mRNAs were more evenly distributed throughout the living cell layers, although even here, stronger hybridizations with the innermost layer were observed. While we cannot exclude the possibility that the expression of these mRNAs may not always coincide with their translation, the protein data (13, 50) are consistent with the RNA localization data presented here. Thus, in contrast to K6, whose mRNA is present under conditions in which the protein is not (47), K5 and K14 seem to be regulated largely at the level of transcription or mRNA stability, and the expression of K5 and K14 mRNAs seems to be coordinately suppressed upon commitment of an epidermal cell to undergo keratinization. This repression in K5 and K14 mRNA levels occurs at the transition of a cell from the basal state to the suprabasal state, and it significantly precedes the loss of transcriptional and metabolic activity of the differentiating epidermal cell. It appears to be one of the first biochemical

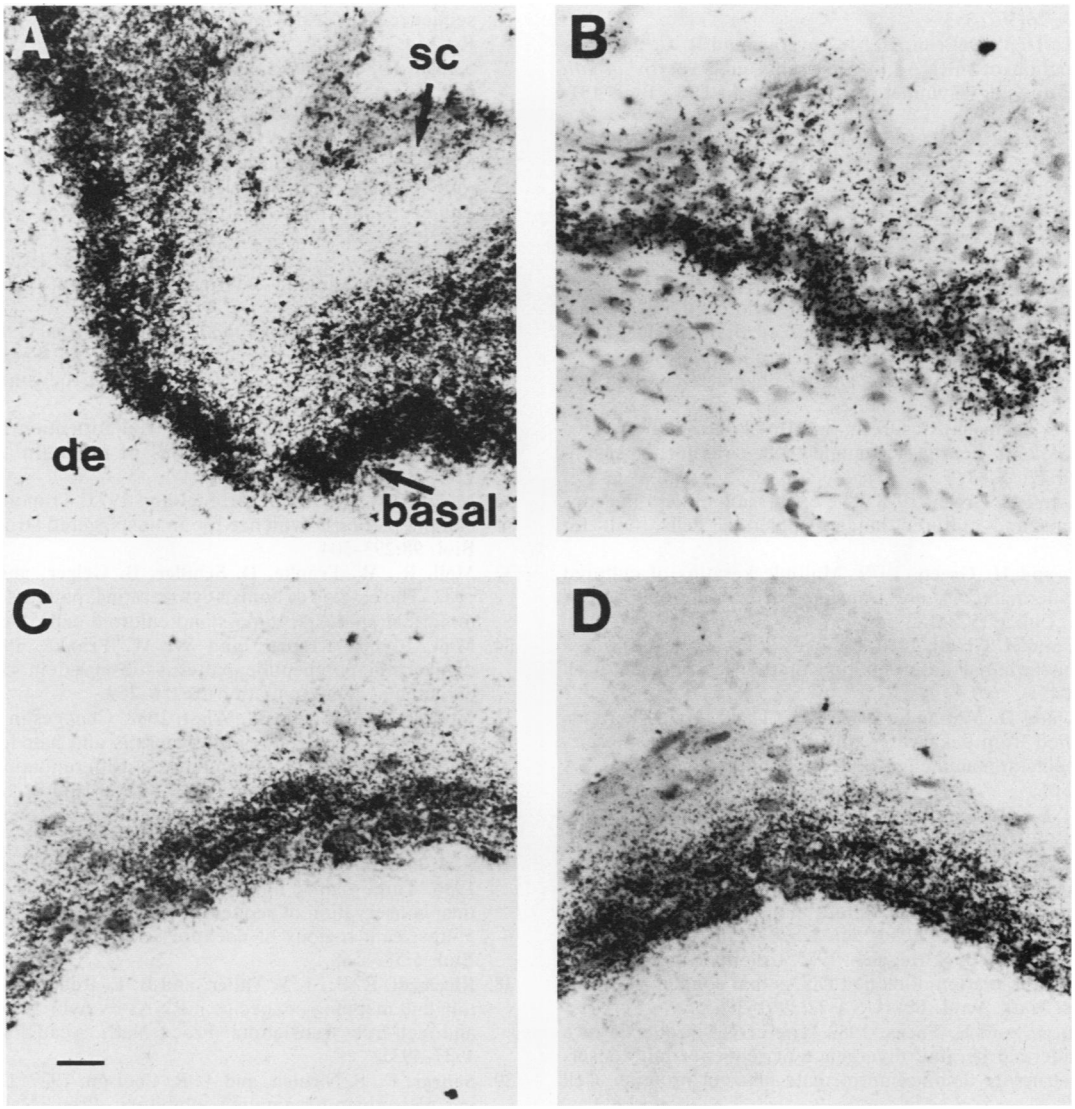

FIG. 5. In situ hybridization of ^{35}S-labeled keratin cRNAs to cross-sections of human epidermis and epidermal cultures. Foreskin tissue (A and B) was fixed in glutaraldehyde and sectioned (5 μm) as described by Cox et al. (5). Confluent cultures of human epidermal cells (C and D) were removed with the enzyme dispase as described by Green et al. (16) and fixed and sectioned via the same method as that used for foreskin. Hybridizations of radiolabeled cRNA probes were followed by RNase treatment and autoradiography. Exposures were for 8 days (A) and for 3 days (B to D). Probes were as follows: A and C, a 1,354-bp *Fnu*DII-*Xba*I fragment of KA-62 encompassing a large portion of the K5-coding region (nucleotide residues 254 to 1,608) and subcloned in the 5′-to-3′ direction into the *Sma*I-*Xba*I site of plasmid pGEM2 (Promega Biotec); B and D, a 1,080-bp *Bst*EII-*Stu*I fragment of KB-2 encompassing a large portion of the K14-coding region (47). de, Dermis; sc, stratum corneum. The innermost basal epidermal layer of the skin is indicated in panel A. Bar, 30 μm. Under the conditions used for hybridization, the keratin probes were monospecific. 3′-Noncoding probes produced similar results, but with higher background levels, owing to the shortness of the probes (K5, 190 bp; K14, 70 bp). A K6 cRNA probe which is highly homologous to K5 revealed grains distributed evenly throughout the epidermis (47). K7, a simple epithelial keratin closely related to K5 (15), revealed almost no cRNA-exposed grains. These controls further verified the specificity of the probes. Only bright-field (not dark-field) microscopy could be conducted, since fluorescing melanin granules were present in the epidermal sections.

events that takes place in the defined program of terminal differentiation.

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