

Sequence and Expression of a Human Type II Mesothelial Keratin

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ABSTRACT Using mRNA from cultured human mesothelial cells, we constructed bacterial plasmids and λ phage vectors that contained cDNA sequences specific for the keratins expressed in these cells. A cloned cDNA encoding keratin K7 (55 kD) was identified by positive hybrid selection. Southern Blot analysis indicated that this sequence is represented only once in the human genome, and Northern Blot analysis demonstrated that the gene encoding K7 is expressed in abundance in cultured bronchial and mesothelial cells, but only weakly in cultured epidermal cells and not at all in liver, colon, or exocervical tissue. The predicted amino acid sequence of this keratin has revealed a striking difference between this keratin and the type II keratins expressed in epidermal cells: whereas all of the epidermal type II keratins thusfar sequenced have long nonhelical termini rich in glycine and serine, this mesothelial type II keratin has amino and carboxy terminal regions that are unusually short and lack the inexact repeats of glycine and serine residues.

The keratins are a family of about 20 different proteins (K1–K19; 40–70 kD) that comprise the 8-nm intermediate filaments in most, if not all, epithelial cells (42). They can be subdivided into two distinct sequence classes, type I and type II (27). The type I keratins are generally small (40–56.5 kD) and acidic (pK_i, 4.5–5.5), whereas the type II keratins are larger (53–67 kD) and more basic (pK_i, 6.5–7.5) (for review, see references 20, 42, and 53). The two types of keratins are frequently expressed as pairs that are differentially expressed in different epithelia and at different stages of differentiation and development (14, 31, 33, 47). At any one time, one to three pairs of keratins are typically expressed, and both types of proteins seem to play an important role in filament assembly (16, 21, 50).

The only keratins that have been sequenced thusfar are the keratins of epidermis (26, 27, 29, 30, 38, 39, 51, 52, 56) and wool (11–13). All of these keratins have a common secondary structure, with a central largely α -helical domain flanked by nonhelical termini (for review, see reference 20). The identification of a keratin according to type is based primarily upon the sequences of the α -helical region. Whereas the two major types of helical sequences share only ~30% homology with each other, individual members of the same type have helical domains that are 50–99% homologous. Although the nonhelical termini are generally much more divergent than are the helical segments of the keratins, they seem to be surprisingly similar for a coordinately expressed pair of type I and type II keratins (12, 26, 27, 30, 31). Thus, for example, epidermal

keratins of both types have termini that contain inexact repeats of Gly-Gly-Gly-X (X = Phe, Tyr, or Leu) interspersed with stretches of serine residues, and in contrast the keratins of wool have termini rich in cysteine. These differences are thought to play a significant role in altering the properties of the resulting 8-nm filaments without affecting their overall structure (12, 27, 51).

The keratins of simple nonkeratinizing epithelia such as the mesothelium differ significantly from the epidermal and wool keratins in their molecular weights, their immunocross-reactivities, their isoelectric mobilities, their solubilities, and their one-dimensional peptide maps (19, 35, 42, 58). Despite such marked differences, the mesothelial keratins can still assemble into filaments that appear to be very similar to the keratin filaments of the epidermis and epidermal appendages (58). To begin to investigate the basis and possible significance of the unique features of the simple epithelial keratins, we have prepared and screened a human mesothelial cDNA library that contain sequences complementary to keratins K7 (55 kD), K8 (53 kD), K18 (44 kD), and K19 (40 kD) (42, 58). In this paper, we report the isolation, characterization, and sequence of a cloned cDNA encoding the 55-kD mesothelial type II keratin, K7.

MATERIALS AND METHODS

Preparation of Human Cell Cultures: Epidermal cell strains were derived from the foreskin of newborns and used in their second to fourth

subculture. Bronchial epithelial cells were cultured from tissue obtained from the small bronchus of the lung. Mesothelial cell strain LP-9 was provided to us from Dr. James Rheinwald (Sidney Farber Cancer Institute), and its culture and derivation from human ascites fluid is described elsewhere (58). All cells were cultured under similar conditions except as noted.

Cells were grown in the presence of lethally mitomycin C-treated 3T3 mouse fibroblasts (45). Mesothelial cell strain LP-9 was grown at high density without the use of 3T3 feeder cells in a 3:1 mixture of Dulbecco's modified Eagle's medium and Ham medium (F12) supplemented with 15% fetal calf serum and 0.4 μ g hydrocortisone/ml (45). Other epithelial cells were grown in the same media with additional supplements of epidermal growth factor (4 ng/ml), 0.1 nM cholera toxin, 20 pM triiodothyronine, human transferrin (5 μ g/ml), and insulin (5 μ g/ml) (43, 44). The medium was changed every 3–4 d and also 12 h before harvesting.

In Vivo Labeling with [³⁵S]Methionine and Extraction of Keratins: Cultures were incubated in one-fourth volume of medium whose methionine concentration was reduced to 3 μ g/ml (one-tenth the normal concentration) and to which [³⁵S]methionine was added to 100 μ Ci/ml. The cells were harvested after 12 h at 37°C. 3T3 feeder cells were selectively removed with EDTA, and the keratins were extracted as previously described (54).

Isolation and Translation of Polyadenylated RNA: Except for mesothelial cells, which were grown to full saturation density, cultures were grown until three-quarters confluent in 50 100-mm culture dishes. Polyadenylated (poly[A]⁺) mRNA was isolated and purified from cells and tissues as previously described (19), and the RNA was translated in an mRNA-dependent rabbit reticulocyte lysate system to which [³⁵S]methionine was added at a concentration of 0.5–1.0 μ M. Translation products were separated by electrophoresis through 8.5% SDS polyacrylamide gels (34).

Isolation of Plasmid and Phage DNA and Purification of cDNA Inserts: Using methods described elsewhere (1, 18, 37, 38), we isolated and purified supercoiled pBR322, pUC9, and λ phage DNA that contained these respective cDNA inserts: (a) KA-1, the 1,680-base insert for the K6a (56 kD) epidermal keratin; (b) KC-1, the 745-base insert for the K7 (55 kD) mesothelial keratin; and (c) KC-2, the 1,319-base insert for K7. The inserts were excised with restriction endonucleases and then separated by 5% polyacrylamide gel electrophoresis. Inserts were purified by electrophoretic elution as described (23).

Positive Hybridization–Translation Assay: Purified, linearized plasmid DNA was denatured and bound to a nitrocellulose filter. Filters were hybridized for 48 h at 41°C with unfractionated poly(A)⁺ RNA from cultured human epithelial cells. Specifically, hybridized RNA was eluted at 65° or 85°C, as indicated. Eluted RNA was translated in vitro in the presence of [³⁵S]methionine, and the translation products were separated by gel electrophoresis as described above.

RESULTS

Construction and Identification of Mesothelial Keratin cDNA Clones

Double stranded cDNA was synthesized (57) from mRNA of cultured human mesothelial cells (LP9) and inserted into the Pst I site of *E. coli* plasmid pUC9 (5). The closed circular plasmids were then used to transform *E. coli* strain TB1 (37). About 1,000 ampicillin-resistant clones with an average insert size of 500-base pair (bp) were isolated.

The clones from the plasmid cDNA library were screened (10, 24) for their ability to cross-hybridize under reduced stringency conditions with ³²P-labeled cDNA prepared from an epidermal type II keratin, K6a (56 kD). This probe was previously shown to cross-hybridize the mRNAs encoding the mesothelial type II keratins K7 (55 kD) and K8 (53 kD) (33). Plasmid DNAs were purified from all positive colonies detected with the epidermal keratin cDNA probe, and the DNAs were subsequently used in a positive hybridization–translation assay (8, 46). Mesothelial mRNA that specifically hybridized

¹ Abbreviations used in this paper: bp, base pair; poly(A)⁺, polyadenylated.

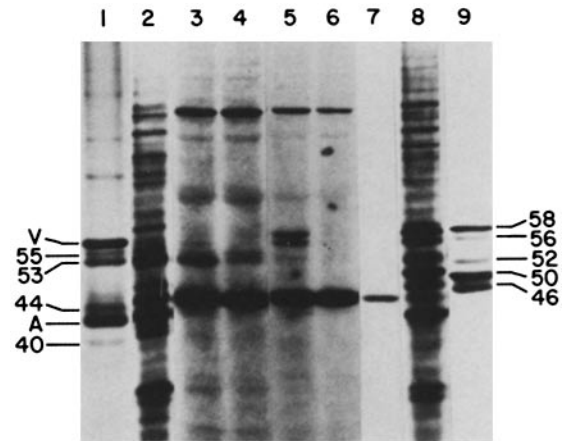


FIGURE 1 Positive identification of the cloned KC-1 insert as a 55-kD (K7) keratin cDNA. The cDNA insert was excised from plasmid pKC-1 by PstI digestion, and the resulting insert fragments were purified. The fragments were denatured and bound to nitrocellulose filters as previously described (33). Each filter was hybridized with either human mesothelial mRNA (lanes 3–4) or human epidermal mRNA (lanes 5–6). After washing away the unhybridized mRNAs at 50°C, specifically bound mRNAs were sequentially eluted at 65°C (lanes 3 and 5) and 85°C (lanes 4 and 6). mRNAs were then translated in vitro in the presence of [³⁵S]methionine, and the protein products were resolved by 8.5% polyacrylamide gel electrophoresis. The gel was fluorographed and exposed to x-ray film. Lane 1, cytoskeletal proteins extracted from [³⁵S]methionine-labeled cultured human mesothelial cells according to the high salt precipitation procedure (58); lane 2, translation products from total mesothelial mRNA; lane 7, mRNA-independent radiolabeled products of the reticulocyte translation system; lane 8, translation products from total human epidermal mRNA; lane 9, immunoprecipitated keratins from [³⁵S]methionine-labeled cultured human epidermal cells. Molecular mass markers are shown at right and left in kilodaltons. V, vimentin; A, actin.

with these cloned cDNAs was eluted and translated in vitro and analyzed by polyacrylamide gel electrophoresis and fluorography.

The translation products of the mRNA selected by one of the putative keratin clones, pKC-1, is shown in Fig. 1. This clone contained a 745-bp insert that hybridized the mRNAs encoding proteins similar in size to the two mesothelial type II keratins K7 and K8 (lane 4). At lower wash temperatures, most of the 53-kD keratin K8 mRNA was eluted (lane 3). Elution of the 55-kD keratin K7 mRNA required higher wash temperatures, which demonstrates that pKC-1 contains the homologous sequence for the K7 mRNA (lane 4). Two-dimensional gel electrophoresis then confirmed the identification of this 55 kD protein translated from pKC-1 hybrid-selected mRNA as the keratin K7: whereas the type II keratins of epidermis have basic isoelectric points (6.5–7.5), the mesothelial keratin K7 is unusually acidic (pK_i, 5.8) (42, 58).

The K7 cDNA also showed cross-hybridization with the mRNAs encoding both epidermal type II keratins, K5 (58 kD) and K6 (56 kD) (lane 5), but these mRNAs were eluted at the lower wash temperatures similar to those required for elution of K8 mRNA. As expected, neither the mRNAs encoding the type I keratins nor the mRNA encoding the type III intermediate filament protein vimentin showed any cross-reactivity with the K7 cDNA.

Construction of a λ gt11 Mesothelial cDNA Library and Isolation of a cDNA Clone that Contains the Near-Complete Coding Region of Keratin K7

Once the keratin clone pKC-1 had been shown to contain a sequence complementary to keratin K7 (55 kD), it was clear that the 745-bp insert could not encompass the entire coding region for this keratin. To increase our chances of obtaining a full-length cDNA clone for K7, we synthesized double stranded mesothelial cDNA according to the procedure of Gubler and Hoffman (25). After the addition of synthetic linkers, the cDNAs were inserted into the EcoRI site of phage vector λ gt11 (59), and *E. coli* strain Y1088 was infected with the packaged λ DNAs. About 1.2×10^5 plaques with an average insert size of 1,000-bp were isolated.

Radiolabeled probe to the K7 sequence in pKC-1 was used to screen the library for additional K7 clones. A number of plaques hybridized with the K7 probe. Of these, one clone λ KC-2, containing a 1,319-bp insert, was chosen for further study. The insert for this clone was isolated (see Materials and Methods) and subcloned into bacteriophage M13mp18 to facilitate subsequent analyses.

Size and Expression of the K7 mRNA in Different Epithelial Tissues

To investigate the occurrence of K7 mRNA in other epithelial cells, we isolated poly(A)⁺ RNAs from a variety of different epithelial tissues and cell cultures. These RNAs can be translated in vitro to yield a number of products in the molecular weight range of 40–70 kD (Fig. 2, left). Some of these translation products have previously been identified as keratins by immunoprecipitation, peptide mapping, and two-dimensional gel electrophoresis (2, 19, 33, 36). To unequivocally demonstrate which of these samples contained K7

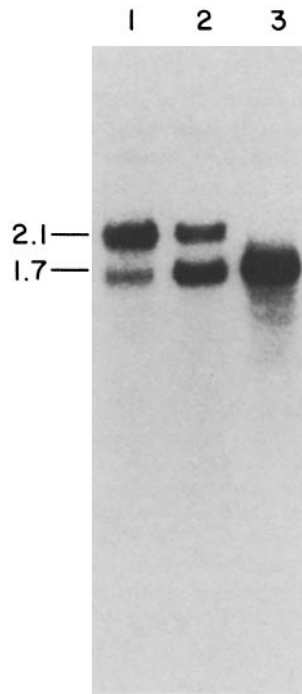
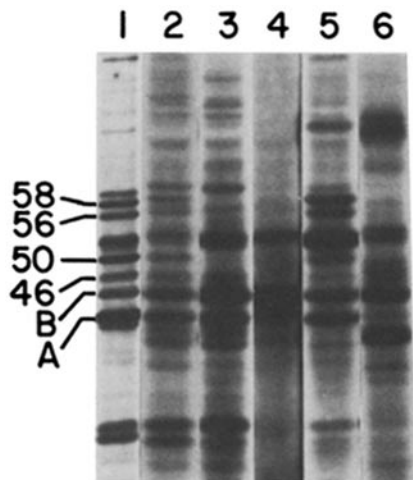


FIGURE 3 Northern Blot analysis at reduced stringency. mRNAs (1 μ g each) from cultured epidermal cells (lane 1), bronchial cells (lane 2), and mesothelial cells (lane 3) were resolved by RNA gel electrophoresis as described (Fig. 2). This time, the nitrocellulose paper was washed at reduced temperature (55°C) to detect crosshybridization of the K7 cDNA probe with other type II keratin mRNAs. Molecular sizes in kilobases are shown at left.

mRNA, we separated the RNAs by electrophoresis on an agarose gel containing 2.2 M formaldehyde (4), transferred them to nitrocellulose paper (51), and hybridized them with K7 cDNA probe under conditions of high stringency (Fig. 2, right). A single and surprisingly small band of 1.7 kb was radiolabeled in cultured cell mRNA from human epidermis (weak, lane 1), bronchus (lane 2), and mesothelium (lane 3), but not from colon (lane 4), exocervix (lane 5), or liver (lane 6). At reduced stringency, a larger band of 2.1 kb also hybridized with the samples from epidermal and bronchial but not from mesothelial cultures (Fig. 3). Since the mRNAs encoding the K5 and K6 keratins have already been shown to be of size

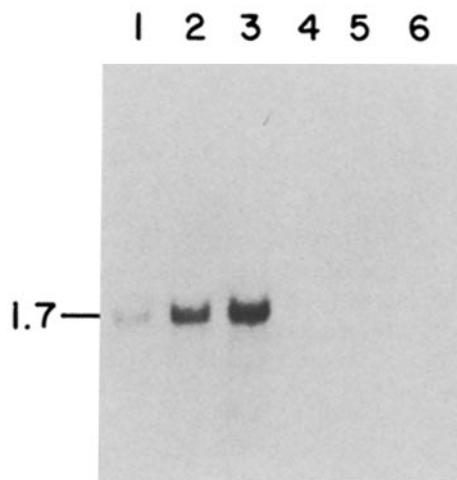


FIGURE 2 Examining the expression of K7 mRNA in different epithelial cells. (Left) Poly (A)⁺ RNAs were isolated as previously described (19). mRNAs were translated in vitro in the presence of [³⁵S]methionine, and the translation products were resolved by polyacrylamide gel electrophoresis. Human mRNAs were from the following sources: lane 1, cultured epidermal cells; lane 2, cultured bronchial epithelial cells; lane 3, cultured mesothelial cells (LP9); lane 4, colon; lane 5, exocervix; lane 6, liver. Molecular weight markers are in kilodaltons at left. B identifies the band resulting from an mRNA-independent artifact of the reticulocyte translation system. A identifies actin. (Right) mRNAs (1 μ g each) were loaded in the sample wells of a 1.2% agarose formaldehyde gel (4). 18S and 28S rRNA from rabbit reticulocytes and 16S and 23S rRNA from *E. coli* were placed in flanking wells as markers. After electrophoresis, the RNAs were transferred onto nitrocellulose paper (55) and then hybridized against ³²P-labeled probe made to the KC-1 insert of K7 cDNA. The blot was washed (0.1% SDS, 0.1 \times SSC) at 68°C and then exposed to film for 12 h. Lanes are same as the gel on the left. Molecular sizes in kilobases are shown at left.

2.1 kb, this additional mRNA band (lanes 1 and 2) most likely represents cross-hybridization of the K7 probe with these RNAs (18). The increased hybridization of probe to the 1.7-kb band in lanes 1-3 probably reflects hybridization of the mRNA encoding the 53-kd keratin (K8), which seems to be similar in size to the 55-kd keratin mRNA. Thus, the mRNAs encoding the two mesothelial type II keratins are much smaller than those encoding the epidermal type II proteins.

There Is a Single Gene That Encodes the K7 Mesothelial Keratin

Human genomic DNA was digested with restriction endonucleases Kpn I or Hind III, and the fragments were resolved by agarose gel electrophoresis. After DNA transfer to nitrocellulose paper (49) and hybridization with ³²P-labeled probe to the K7 cDNA from pKC-1, it was discovered that a single major band of ~4.2 kb became radiolabeled (Fig. 4). This finding suggests that there is only a single gene that encodes the K7 keratin. In contrast, there seem to be multiple genes that encode the K6 keratin and at least two of these are coordinately expressed in cultured human epidermal cells (18, 56).

The Sequence of the cDNA That Encodes Mesothelial Keratin K7

To obtain the amino acid sequence for the mesothelial keratin K7, we determined the nucleotide sequences of both pKC-1 and λKC-2. Fig. 5 illustrates the strategy that was used for sequencing. Preliminary restriction map analysis of the two clones provided a number of hexanucleotide sites that were then used to subclone insert fragments into phage vectors

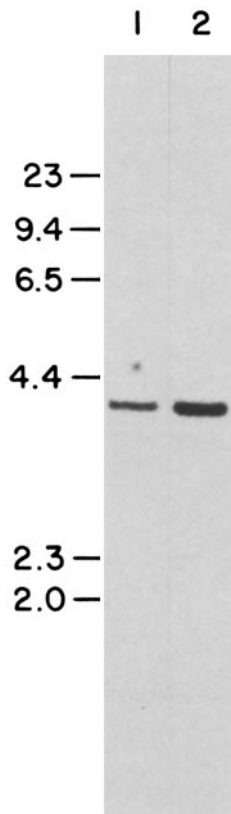


FIGURE 4 A single gene encodes the mesothelial keratin K7. DNA (5 μg) from cultured human diploid fibroblasts was digested with a fivefold excess of each of two restriction endonucleases: KpnI (lane 1) and HindIII (lane 2). Fragments were fractionated by electrophoresis through a 0.8% agarose gel and transferred to nitrocellulose paper (49). The blots were hybridized with ³²P-labeled probes from pKC-1 K7 cDNA. Molecular weights in kilobases are shown at the left. Note that although the pKC-1 cDNA does not have a HindIII site, there is a KpnI site located 135 bp from the 5' end of this clone. It is expected that the 4.2-kb KpnI fragment of the human genome is the fragment that contains the remaining 610 bp of the pKC-1 clone that are located 3' downstream from this site.

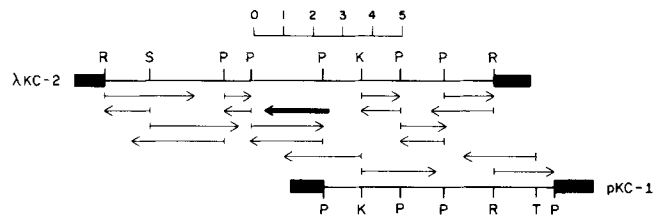


FIGURE 5 DNA sequencing strategies for the human mesothelial K7 keratin cDNA inserts KC-1 and KC-2. A restriction map of the KC-2 insert (thin line) flanked by λ sequences (heavy lines) is shown at the top. A restriction map of the KC-1 insert (thin line) flanked by pUC9 sequences (heavy lines) is shown at the bottom. The direction from left to right is the 5'-to-3' direction of the mRNA strand, and the positions of all recognition sites for each enzyme are indicated: P, Pst I; R, Eco RI; T, Tha I; S, Sac I; K, Kpn I. Arrows designate the direction and the extent of DNA sequence determination, which was conducted according to Sanger (48) and Maxam and Gilbert (37). The thick arrow represents sequence information obtained from KC-2 using a synthetic oligonucleotide primer prepared to a sequence near the 5' end of KC-1: 5' GACAGCACCACAGATGT3'.

M13mp18 and M13mp19 for sequencing (48). In the 536 bp of overlapping sequence, the two clones showed 100% nucleotide homology.

The nucleotide and predicted amino acid sequence of the mesothelial keratin K7 cDNA is shown in Fig. 6 aligned with the complete K6b (56 kD) epidermal type II sequence previously obtained from the genomic sequence (56). A total of 1,516 bp of the K7 mRNA is contained in the two cloned inserts. Given the estimated size of 1,700 bp for the K7 mRNA (Fig. 2), approximately 90% of its sequence has been determined. A large open reading frame is present that encompasses the sequence beginning at the 5' end of the KC-2 cDNA and extending to 49 bp upstream from the 3' end of the KC-1 cDNA. Neither the classic polyadenylation signal AATAAA nor the poly A tract are present, suggesting that a part of the 3' noncoding sequence of KC-1 was lost during the S1 endonuclease step of the cloning procedure (57). Since an EcoRI site is located 5' upstream from the putative TGA stop codon, this sequence was also lost from KC-2, in this case during the synthetic linker-ligation step (37, 59).

No putative translation initiation site was found in the open reading frame, despite the presence in a different reading frame of an ATG sequence 62 nucleotides 3' downstream from the 5' end of KC-2. Although this ATG does not seem to be the correct initiation site for translation of the K7 mRNA, we cannot exclude this possibility, because the 5' end region is unusually abundant in G-C rich sequences. These sequences could give rise to hidden nucleotide compressions in the sequence analyses of both strands. If indeed the true translation initiation site of the K7 mRNA is located 5' upstream from the end of the KC-2 sequence as indicated in Fig. 6, it must be very close to this end, since we estimate that there are only about 200 nucleotides of missing sequence information, some of which must be 5' and 3' untranslated sequence. Therefore, the predicted amino acid sequence (491 residues) presented in Fig. 6 must represent most of the coding portion of the K7 keratin. The putative polypeptide size of the near complete sequence is 54,115 D, which agrees well with that determined previously by SDS polyacrylamide gel analysis (19).

The predicted amino acid sequence clearly identifies the

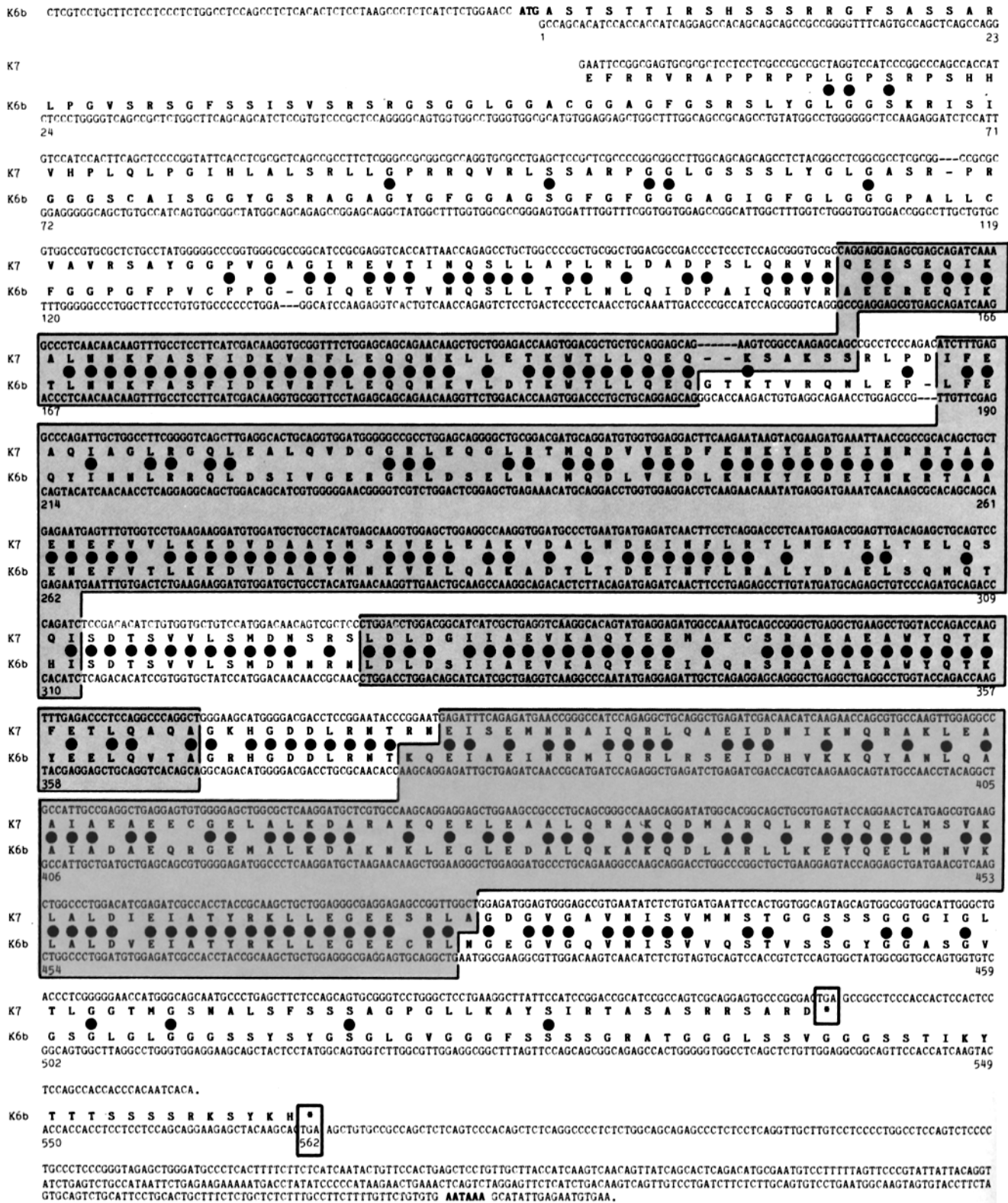


FIGURE 6 The complete nucleotide and predicted amino acid sequence of the mesothelial K7 cDNAs KC-1 and KC-2 and their comparison with the epidermal K6b cDNA. The sequence is shown in the 5'-to-3' direction of the mRNA strand. The amino acid sequences of the K7 cDNA (this paper) and K6b (56) were aligned for optimal homology. The numbers mark the positions of the amino acid residues of the K6b cDNA as described previously (56). The solid circles indicate positions of identity in the amino acid sequence. The two sequences share 56% homology at the amino acid sequence level and 68% at the nucleic acid sequence level. The gray boxes mark the boundaries of the predicted α -helical domains in the two sequences (27, 56). Throughout these domains are the heptad repeats of hydrophobic residues, which identify the portions of the polypeptides that are involved in coiled-coil interactions with a second keratin coordinately expressed in their respective tissues.

protein encoded by the KC-1 and KC-2 cDNAs as a keratin. The α -helical segments (encompassed by the gray boxes in Fig. 6), predicted by the methods of Chou and Fasman (6, 7) and Garnier et al. (22), appear at positions that are nearly identical to the helical domains of the K6b epidermal type II keratin. Within these helical domains, the K7 amino acid sequence shares far more homology to the epidermal K6b type II keratin (73%; reference 56) than to the epidermal K14 type I keratin (43%; references 26, 38, and 39). Although this relation unequivocally demonstrates that mesothelial keratin K7 belongs to the type II keratin family, it is a distant member, considering that other epidermal type II keratins share 93–99% homology with each other throughout the α -helical domains (27, 29, 30, 52, 56). The only other type II keratin sequenced to date that shows a greater level of sequence deviation in this region is the wool type II keratin, which shares only 63–65% homology with the epidermal type II keratins (11–13).

Despite the marked divergence in sequence, the general structural features of the mesothelial keratin K7 are similar to those of the epidermal keratins. Indeed, many of the amino acid residue differences are conservative ones, e.g., Arg \rightarrow Lys or Asn \rightarrow Gln. As such, the predicted α -helical domains show largely the same boundaries (marked by the gray boxes in Fig. 6). In addition, throughout the α -helical segments are the characteristic heptad repeats of hydrophobic residues that signify the portions of the keratin polypeptides involved in coiled-coil formation (40, 41). The only major difference between the helical domains of the epidermal keratins and those of K7 is that the K7 sequence in these regions is less basic, which may contribute to the more acidic isoelectric focusing point of K7 over other type II keratins (42).

The K7 Mesothelial Keratin Has Unusual Amino and Carboxy Terminal Segments

The nonhelical amino and carboxy termini of K7 are substantially different both in sequence and in size from K6b and from other epidermal type II keratins. Whereas the epidermal keratins typically have nonhelical terminal segments abundant in inexact repeats of Gly-Gly-Gly-X (where X = Phe, Tyr, Leu, or Ile) that are interspersed with 4–6 serine residues, the corresponding regions of the mesothelial keratin bear almost no resemblance. Only one Gly-Gly-Gly-Ile sequence and three stretches of only three serine residues are present in the amino and carboxy terminal sequences combined. In the amino terminal segment, the glycine and serine rich repeats are replaced with leucine- and proline-rich sequences interspersed with single glycine and serine residues. Similarly, the serine and glycine residues in the carboxy terminal segment of K7 are more dispersed than they are in this region of the epidermal keratins.

Perhaps the most striking feature of the mesothelial keratin K7 is the unusually short length of the amino and carboxy terminal segments. In contrast to the nonhelical carboxy termini of the epidermal keratins, which vary from 88 to 150 residues (27, 29, 30, 52, 56), the carboxy terminal end of K7 is only 66 residues in length. Moreover, if the putative amino terminus of K7 is not much more than the 108 amino acids shown in Fig. 6, this is shorter than the corresponding region of the epidermal keratins, which are 150–190 residues.

DISCUSSION

Probing the Expression of the mRNA That Encodes the K7 Keratin

Human mesothelial cells synthesize four major keratins, K7 (55 kD), K8 (53 kD), K18 (44 kD), and K19 (40 kD) (58). The 55-kD type II keratin (K7) differs from other type II keratins in that no clear evidence for a constant partner type I keratin has ever been established (14, 42). Rather, one or the other or both of the 44-kD (K18) and the 40-kD (K19) keratins were always co-expressed with K7. Moreover, whereas the 53-kD keratin K8 is frequently expressed in the absence of K7, it seems that K7 expression is dependent upon the coordinate expression of K8.

The isolation of a cloned cDNA for K7 provides the first specific probe for this keratin, which has enabled us to begin to examine its sequence, structure, and expression in different human epithelia. The specificity of the probe is evident from several different criteria: (a) under stringent conditions, no cross-hybridization was detected between radiolabeled K7 probe and colon mRNA, even though colon epithelium expresses all three other keratins found in mesothelial cells (K8, K18, and K19) (42); (b) under stringent conditions, K7 cDNA shows no cross-hybridization with the major epidermal type II keratins, K5 and K6; and (c) in a positive hybridization translation assay under stringent conditions, K7 mRNA is selected with KC-1 and KC-2 cDNAs.

Using our specific K7 probe, we have confirmed the presence of K7 in cultured mesothelial (58) and bronchial (3, 42) epithelial cells, as well as its absence in colon, liver, and exocervix (42). In contrast to previous reports (42, 58), we have detected low levels of K7 expression in cultured human epidermal cells, thereby illustrating the increase in sensitivity of K7 detection obtained with the cloned cDNA probe. Moreover, during the course of screening our mesothelial cDNA library, we obtained a cloned cDNA encoding the 56-kD (K6) keratin expressed in abundance in cultured human epidermal cells, but previously thought to be absent in cultured mesothelial cells (58). Thus, our results suggest that there may be a very low level expression of epidermal keratins in mesothelial cells and a low level of expression of mesothelial keratins in epidermal cells. The possible significance of these findings is presently unknown.

The Unusual Sequence of the K7 Keratin May Influence the Properties of the 8-nm Filaments in Simple Epithelia

A single gene encodes the K7 keratin expressed in human mesothelial cells. This gene encodes an mRNA that is smaller than most type II keratin mRNAs. We have determined the sequence of 90% of the K7 mRNA, which appears to include most, if not all of the coding portion of the transcript. The predicted amino acid sequence for the 55-kD keratin has revealed that the small size of the K7 mRNA is partly due to unusually short amino and carboxy terminal segments of the polypeptide chain. All four predicted α -helical domains in the central region of the polypeptide chain are highly similar in structure to those of the keratins of epidermis and wool. Since the helical domains of the keratins dictate the coiled-coil structure of the intermediate filament subunits, the keratin

filament in simple epithelia is probably not substantially different from that of other epithelial cells.

Although the short nonhelical terminal segments of K7 may not alter the overall structure of the mesothelial filaments, they are likely to play a role in determining the unique properties of these filaments. In the case of the epidermal keratins, mild chymotryptic digestion selectively removes the nonhelical end segments and renders the subunits incompetent for filament formation (50, 51). These results suggest that the ends of keratin polypeptides may play an important role in end-to-end and in lateral interactions of the coiled-coil subunits. Interestingly, for both the wool and the mesothelial keratins, it seems to be the end terminal sequences that show the most significant divergence from the epidermal keratins.

The differences in the nonhelical terminal regions of the three tissue-specific groups of keratins could certainly account for the variation in solubility among the 8-nm keratin filaments in different tissues (17, 58). In addition, such features as flexibility, tensile strength, and ability to interact with other cellular components may be affected. Although we expect that most of the variation in the properties of the keratin filaments can be attributed to the divergent nonhelical termini, it is possible that the less marked sequence variations in the α -helical regions of the keratins may also contribute to these features. The more acidic nature of the α -helical domains of K7 may be such an example. As we obtain more sequence information for the other mesothelial keratins, we hope to be able to define more precisely the physiological differences between the 8-nm filaments of simple epithelia and those of the epidermis and epidermal appendages. The sequence of the K7 gene and the genes encoding the other mesothelial keratins should also provide us with the necessary prerequisites to begin to probe the molecular mechanisms underlying the tissue-specific expression of these unusual keratin genes in simple human epithelial tissues.

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REFERENCES

- Benton, W. D., and R. W. Davis. 1977. Screening λ gt recombinant clones by hybridization to single plaques in situ. *Science (Wash. DC)*. 196:180-182.
- Bladon, P. T., P. E. Bowden, W. J. Conliffe, and E. J. Wood. 1982. Prekeratin biosynthesis in human scalp epidermis. *Biochem. J.* 208:179-187.
- Blobel, G. A., R. Moll, W. W. Franke, and I. Vogt-Moykopf. 1984. Cytokeratins in normal lung and lung carcinomas. *Virchows Arch. Cell Pathol.* 45:407-429.
- Boedtker, H. 1971. Conformation independent molecular weight determinations of RNA by gel electrophoresis. *Biochem. Biophys. Acta.* 240:448-453.
- Chang, A. C. Y., J. H. Nunberg, R. J. Kaufman, H. A. Erlich, R. T. Schimke, and S. N. Cohen. 1978. Phenotypic expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase. *Nature (Lond.)*. 275:617-624.
- Chou, P. Y., and G. D. Fasman. 1978. Prediction of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45-148.
- Chou, P. Y., and G. D. Fasman. 1979. Prediction of β -turns. *Biophys. J.* 26:367-383.
- Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. 1980. Number and evolutionary conservation of α - and β -tubulin and cytoplasmic β - and γ -actin genes using specific cloned cDNA probes. *Cell.* 20:95-105.
- Connell, N. D., and J. G. Rheinwald. 1983. Regulation of the cytoskeleton in mesothelial cells: reversible loss of keratin and increase in vimentin during rapid growth in culture. *Cell.* 34:245-253.
- Craig, E. A., B. J. McCarthy, and S. C. Wadsworth. 1979. Sequence organization of two recombinant plasmids containing genes for the major heat shock-induced protein of *D. melanogaster*. *Cell.* 16:575-588.
- Crewther, W. G., L. M. Dowling, and A. S. Inglis. 1980. Amino acid sequence data from a microfibrillar protein of α -keratin. *Proc. Quinquennial Intl. World Textile Conf., 6th, Pretoria.* 2:79-91.
- Crewther, W. G., L. M. Dowling, P. M. Steinert, and D. A. D. Parry. 1983. Structure of intermediate filaments. *Int. J. Biol. Macromol.* 5:267-274.
- Dowling, L. M., D. A. D. Parry, and L. G. Sparrow. 1983. Structural homology between α -keratin and intermediate filament proteins desmin and vimentin. *Biochim. Biophys. Res. Commun.* 113:73-78.
- Eichner, R., P. Bonitz, and T.-T. Sun. 1984. Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. *J. Cell Biol.* 98:1388-1396.
- Fitzgerald, M., and T. Shenk. 1981. The sequence 5'-AAUAAA-3' forms part of the recognition site for polyadenylation of late SV40 mRNAs. *Cell.* 24:251-260.
- Franke, W. W., D. L. Schiller, R. Hatzfeld, and S. Winter. 1983. Protein complexes of intermediate-sized filaments: melting of cytokeratin complexes in urea reveals different polypeptide separation characteristics. *Proc. Natl. Acad. Sci. USA.* 80:7113-7117.
- Fraser, R. D. B., T. P. MacRae, and G. E. Rogers. 1972. Keratins: their composition, structure and biosynthesis. C. C. Thomas, Springfield, IL. 30-120.
- Fuchs, E., S. M. Coppock, H. Green, and D. W. Cleveland. 1981. Two distinct classes of keratin genes and their evolutionary significance. *Cell.* 27:75-84.
- Fuchs, E., and H. Green. 1980. Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell.* 19:1033-1042.
- Fuchs, E., and I. Hanukoglu. 1983. Unraveling the structure of the intermediate filament. *Cell.* 34:332-334.
- Fuchs, E., and D. Marchuk. 1983. Type I and type II keratins have evolved from lower eukaryotes to form the intermediate filaments in mammalian skin. *Proc. Natl. Acad. Sci. USA.* 80:5857-5861.
- Garnier, J., D. J. Osguthrope, and B. Robson. 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* 120:97-120.
- Girvitz, S. C., S. Bacchetti, A. J. Rainbow, and F. L. Graham. 1980. A rapid and efficient procedure for the purification of DNA from agarose gels. *Anal. Biochem.* 106:492-496.
- Grunstein, M., and D. S. Hogness. 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA.* 72:3961-3965.
- Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. *Gene.* 25:263-269.
- Hanukoglu, I., and E. Fuchs. 1982. The cDNA sequence of a human epidermal keratin: divergence of sequence by conservation of structure among intermediate filament proteins. *Cell.* 31:243-252.
- Hanukoglu, I., and E. Fuchs. 1983. The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. *Cell.* 33:915-924.
- Jackson, B. W., C. Grund, E. Schmid, K. Burke, W. W. Franke, and K. Illmensee. 1980. Formation of cytoskeletal elements during mouse embryogenesis. I. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation embryos. *Differentiation.* 17:161-179.
- Johnson, L. D., W. W. Idler, X.-M. Zhou, D. R. Roop, and P. M. Steinert. 1985. Structure of a gene for the human epidermal 67-kDa keratin. *Proc. Natl. Acad. Sci. USA.* 82:1896-1900.
- Jorcano, J. L., J. K. Franz, and W. W. Franke. 1984. Amino acid sequence diversity between bovine epidermal cytokeratin polypeptides of the basic (type II) subfamily as determined from cDNA clones. *Differentiation.* 28:155-163.
- Jorcano, J. L., T. M. Magin, and W. W. Franke. 1984. Cell type-specific expression of bovine keratin genes as demonstrated by the use of complementary DNA clones. *J. Mol. Biol.* 176:21-37.
- Jorcano, J. L., M. Rieger, J. K. Franz, D. L. Schiller, R. Moll, and W. W. Franke. 1984. Identification of two types of keratin polypeptides within the acidic cytokeratin subfamily I. *J. Mol. Biol.* 179:257-281.
- Kim, K.-H., J. G. Rheinwald, and E. Fuchs. 1983. Tissue specificity of epithelial keratins: differential expression of mRNAs from two multigene families. *Mol. Cell. Biol.* 3(4):495-502.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680-685.
- LaRocca, P. J., and J. G. Rheinwald. 1984. Coexpression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma *in vivo* and in culture. *Cancer Res.* 44:2991-2999.
- Magin, T. M., J. L. Jorcano, and W. W. Franke. 1983. Translational products of mRNAs coding for non-epidermal cytokeratins. *EMBO (Eur. Mol. Biol. Organ.) J.* 2(8):1387-1392.
- Maniatis, T., E. F. Fritsch, and J. Sambrook, eds. 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY. 250-252.
- Marchuk, D., S. McCrohon, and E. Fuchs. 1984. Remarkable conservation of structure among intermediate filament genes. *Cell.* 39:491-498.
- Marchuk, D., S. McCrohon, and E. Fuchs. 1985. Complete sequence of a gene encoding a human type I keratin: sequences homologous to enhancer elements in the regulatory region of the gene. *Proc. Natl. Acad. Sci. USA.* 82:1609-1613.
- McLachlan, A. D. 1978. Coiled-coil formation and sequence regularities in the helical regions of α -keratin. *J. Mol. Biol.* 124:297-304.
- McLachlan, A. D., and M. Stewart. 1975. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. *J. Mol. Biol.* 98:293-304.
- Moll, R., W. W. Franke, D. L. Schiller, B. Geiger, and R. Krepler. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell.* 31:11-24.
- Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 6:331-343.
- Rheinwald, J. G., and H. Green. 1977. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature (Lond.)*. 265:421-424.

45. Rheinwald, J. G. 1980. Serial cultivation of normal human epidermal keratinocytes. *Methods Cell Biol.* 21A:229-254.
46. Ricciardi, R. P., J. S. Miller, and B. E. Roberts. 1979. Purification and mapping of specific mRNAs by hybridization-selection and cell-free translation. *Proc. Natl. Acad. Sci. USA.* 76:4927-4931.
47. Roop, D. R., P. Hawley-Nelson, C. K. Cheng, and S. H. Yuspa. 1983. Keratin gene expression in mouse epidermis and cultured epidermal cells. *Proc. Natl. Acad. Sci. USA.* 80:716-720.
48. Sanger, F., A. R. Coulson, B. G. Barrel, A. J. H. Smith, and B. A. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* 143:161-178.
49. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
50. Steinert, P. M., W. W. Idler, and B. Zimmerman. 1976. Self-assembly of bovine epidermal keratin filaments *in vitro*. *J. Mol. Biol.* 108:547-567.
51. Steinert, P. M., R. H. Rice, D. R. Roop, B. L. Trus, and A. C. Steven. 1983. Complete amino acid sequence of a mouse epidermal keratin subunit and implications for the structure of intermediate filaments. *Nature (Lond.)*. 302:794-800.
52. Steinert, P. M., D. A. D. Parry, E. L. Racoosin, W. W. Idler, A. C. Steven, B. L. Trus, and D. R. Roop. 1984. The complete cDNA and deduced amino acid sequence of a type II mouse epidermal keratin of 60,000 Da: analysis of sequence differences between type I and type II keratins. *Proc. Natl. Acad. Sci. USA.* 81:5709-5713.
53. Sun, T.-T., R. Eichner, A. Schermer, D. Cooper, W. G. Nelson, and R. A. Weiss. 1984. Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: a unifying model. *In* The Cancer Cell. Volume I. The Transformed Phenotype. A. Levine, W. Topp, G. Van Woude, and J. D. Watson, editors. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY. 169.
54. Sun, T.-T., C. Shih, and H. Green. 1978. Keratin filaments of cultured human epidermal cells. Formation of intermolecular disulfide bonds during terminal differentiation. *J. Biol. Chem.* 253:2053-2060.
55. Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201-5205.
56. Tyner, A. L., M. J. Eichman, and E. Fuchs. 1985. The complete sequence of a type II keratin gene expressed in human epidermis: conservation of structure among all intermediate filament genes. *Proc. Natl. Acad. Sci. USA.* 82:4683-4687.
57. Wickens, M. P., G. N. Buell, and R. T. Schimke. 1978. Synthesis of double-stranded DNA complementary to lysozyme, ovomucoid and ovalbumin mRNAs. *J. Biol. Chem.* 253:2483-2495.
58. Wu, Y.-J., L. M. Parker, N. E. Binder, M. A. Beckett, J. H. Sinard, C. T. Griffiths, and J. G. Rheinwald. 1982. The mesothelial keratins: a new family of cytoskeletal proteins identified in cultured mesothelial cells and nonkeratinizing epithelia. *Cell.* 31:693-703.
59. Young, R. A., and R. W. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. USA.* 80:1194-1198.