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Tissue Specificity of Epithelial Keratins: Differential Expression of mRNAs from Two Multigene Families

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Human epithelial cells cultured from stratified and simple squamous tissues all produce keratins of 40,000 to 58,000 daltons, but within this range the number and sizes vary with different epithelial cells. We have shown that this tissue-specific variation in the keratins is not due to posttranslational modification or processing, but rather to the differential expression of a family of heterogeneous but closely related mRNAs. All of these epithelial keratin mRNAs can be further grouped into two distinct subfamilies by their ability to hybridize with either of two cloned epidermal keratin cDNAs. All of the keratin mRNAs hybridize to one or the other, but not both, of the two cloned cDNAs. However, the mRNAs within each group hybridize with varying degrees of stringency, indicating that they are of similar but not identical sequence. Both types of keratin mRNAs are always expressed in every epithelial cell line studied, suggesting that filament assembly is dependent on the presence of both types of keratins. Within each of these two groups, the slight sequence differences in each class may reflect subtle tissue-specific variations in the structural and functional requirements of the epithelial cytoskeleton.

The keratins are a family of at least 10 closely related proteins (40 to 70 kilodaltons [kd]) that form structurally indistinguishable 8-nm cytoplasmic filaments in most vertebrate epithelial cells (1, 4, 6, 36, 40). Typically, only two to six keratins are expressed by a particular cell, and this subset varies with cell type (10, 15, 23, 41, 43, 46). For some epithelial cells, e.g., epidermal and conjunctival, the subsets of keratins produced are partially overlapping (16, 41). For other cell types, e.g., epidermal and mesothelial, the subsets are completely unique (46).

All epithelial cells appear to make some keratins of intermediate size (46 to 58 kd), but expression of the larger (63 to 67 kd) and the smaller (40 to 45 kd) keratins is more restricted. The large keratins are abundant only in differentiating epidermal cells and are probably associated with stratum corneum formation (7, 13, 15, 35). The smaller keratins are present in conjunctival cells (16, 41), the intestinal epithelium (10), mesothelial cells (46), and many simple and glandular epithelia (24), but they are not prominent in epidermal cells, esophageal cells, or epithelial cells of the oral cavity (10, 15, 23).

The significance of the multiplicity of keratins and their differential expression remains largely undetermined. For the keratins of the epidermis, it is clear that the polypeptides are not related solely by posttranslational processing or modification. Although the individual polypeptides are similar, they can be distinguished both immunologically and biochemically, and most are translated from different mRNAs (14, 15, 34). Recently, workers in our laboratory showed that these epidermal keratin mRNAs can be grouped into two distinct subclasses on the basis of their sequence (12). In positive hybridization-translation assays with cloned cDNAs prepared from the four major keratin mRNAs of cultured human epidermal cells, one class of keratin cDNA sequences removed from total human epidermal mRNA a fraction that translated into 46- and 50-kd keratins, whereas the other class selected mRNAs that translated into 56- and 58-kd keratins. Both classes of sequences are encoded by separate multigene families (about 10 genes each), which are coordinately conserved throughout vertebrate evolution. The extent of homology among the members within each keratin multigene family was not initially determined.

To explore the nature of tissue-specific differences in keratin gene expression, we extended our studies to the keratins of other human epithelial cells. Recently, we identified four nonepidermal keratins as the subset of keratins synthesized by cultured mesothelial cells (46) and six

keratins as the subset synthesized by cultured conjunctival cells (16, 41). In the present paper, we show that discrete mRNAs exist for all of these keratins (40 to 58 kd). Our results indicate that each of these different keratin mRNA sequences is homologous to one or the other, but not both, of the two classes of cloned keratin cDNAs that we had previously derived from human epidermal mRNA (12).

Even the mRNAs encoding the simple squamous mesothelial keratins (40, 44, 53, and 55 kd) fell into these two distinct classes based on their ability to hybridize with the cloned epidermal cDNAs for the 46- to 50-kd and the 56- to 58-kd keratins. For all epithelial cells examined, in no case did we find cells that produced one class of keratin mRNA sequence but not the other. Our findings suggest that the presence of these two classes of keratin mRNA sequences may be a universal property among vertebrate epithelial cells. Within each class, the mRNAs are similar but not identical, and their expression appears to be tailored to cell type and relative stage of differentiation.

MATERIALS AND METHODS

Preparation of cell cultures. Human epidermal cell strains were derived from the foreskin of newborns and used in their second to fourth subculture. Human conjunctival keratinocytes were derived from an autopsy of a newborn and used in their third subculture. The derivation of SCC-15 and other established cell lines from human squamous cell carcinomas of the epidermis and tongue is described elsewhere (28). The derivation of mesothelial cell strain LP-9 from human ascites fluid is also described elsewhere (46). All cells were cultured under similar conditions except as noted.

Cells were grown in the presence of lethally mitomycin-treated 3T3 mouse fibroblasts (27, 29). Mesothelial cell strain LP-9 was grown at high density without the use of 3T3 feeder cells in a 3:1 mixture of Dulbecco modified Eagle medium and Ham medium (F12) supplemented with 20% fetal calf serum and 0.4 μg of hydrocortisone per ml (27, 30). Other epithelial cells were grown in the same media with additional supplements of epidermal growth factor (4 ng/ml) prepared by the method of Savage and Cohen (32), 0.1 nM cholera toxin, 20 pM triiodothyronine, human transferrin (5 μg/ml), and insulin (5 μg/ml) (2, 22). The medium was changed every 3 to 4 days and also 12 h before the cells were harvested.

In vivo labeling with [^{35}S]methionine and extraction of keratins. Cultures were incubated in one-fourth volume of medium whose methionine concentration was reduced to 3 μg/ml (one-tenth the normal concentration) and to which [^{35}S]methionine was added to 100 μCi/ml. The cells were harvested after 12 h at 37°C. 3T3 feeder cells were selectively removed with EDTA (39), and the keratins were extracted as previously described (16).

Isolation and translation of poly(A)$^+$ 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)$^+$] RNA was isolated and purified from these cells by the guanidine procedure of Strohman et al. (38), modified as described by Fuchs and Green (14). Poly(A)$^+$ RNA was translated in an mRNA-dependent rabbit reticulocyte lysate system (26) to which [^{35}S]methionine was added at a concentration of 0.5 to 1.0 μM.

Isolation of plasmid DNA and purification of cDNA inserts. As described elsewhere (12), we isolated and purified supercoiled hybrid pBR322-cDNA plasmids containing the following cDNA inserts: (i) KA-1, the 1,680-base insert for the 56-kd epidermal keratin; (ii) KB-2, the 1,450-base insert for 50-kd epidermal keratin; and (iii) A-1, the 880-base insert for cytoskeletal actin. The inserts were excised with restriction endonucleases and then separated by 5% polyacrylamide gel electrophoresis. Inserts were then purified by electrophoretic elution as described by Girvitz et al. (17).

Positive hybridization-translation assay. Purified, linearized plasmid DNA was denatured and bound to a nitrocellulose filter by the method of Kafatos et al. (19). 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, 85, or 100°C, as indicated. Eluted RNA was translated in vitro in the presence of [^{35}S]methionine (26), and the translation products were fractionated by gel electrophoresis.

Gel electrophoresis. Electrophoresis of DNA was performed with vertical slab gels cast from either 0.8% agarose or 5% polyacrylamide containing 40 mM Tris-hydrochloride, 20 mM sodium acetate, and 2 mM EDTA (pH 8.1).

One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins was carried out with 8.5% polyacrylamide (30:0.8, acrylamide-bisacrylamide) gels by the procedure of Laemmli (20). Two-dimensional electrophoresis of proteins was carried out as described by O'Farrell (25). Isoelectric focusing in the first dimension was with a 4.5 to 7.0 pH gradient, and sodium dodecyl sulfate-polyacrylamide (8.5%) gel electrophoresis was used for the second dimension. Protein gels were developed by fluorography (3) and autoradiography with X-Omat R film.

RESULTS

Growth of human epithelial cells in culture and their synthesis of keratins. Four types of cultured human epithelial cells were chosen for study: epidermal cells; SCC-15 cells, an established epithelial cell line from a human squamous cell carcinoma; conjunctival cells; and peritoneal mesothelial cells. These four cell types have all been shown to retain many of their morphological and biochemical properties in culture. Thus, the first three cell types, which were cultured from different stratified squamous epithelia, stratify in culture, whereas the mesothelial cells, which were cultured from a simple squamous epithelium, grow in a monolayer at saturation density. Moreover, the different patterns of ker-

atins produced by these cells are similar to those in the tissues from which these cells originate. Cultured epidermal cells synthesize four major (46, 50, 56, and 58 kd) and one minor keratin (52 kd). The SCC-15 cells from a squamous cell carcinoma of the tongue synthesize all of the keratins made by the epidermal cells, but in addition produce significant levels of a 40-kd keratin (45). Cultured conjunctival cells also produce keratins of 40, 46, 50, 52, 56, and 58 kd, although the relative amounts of these polypeptides are somewhat different from those present in the squamous cell carcinoma line (41). Cultured mesothelial cells make a novel set of keratins of 40, 44, 53, and 55 kd (46). (Note that the 53-kd mesothelial keratin reported here is identical to the 52 K_M keratin described previously [46].) A 56-kd protein, identified as vimentin, is also abundant in mesothelial cells and is coextracted with keratin (46).

Isolation and translation of mRNA. Poly(A)$^+$ RNA was isolated from the four types of human epithelial cells listed above. With the guanidine-vanadyl ribonucleoside method described previously (14, 15), approximately 40 μg of total RNA per 10^6 cells could be recovered from the normal cultured keratinocytes, whereas 60 μg of RNA per 10^6 cells was typically recovered from the cell lines derived from human squamous cell carcinomas. For all epithelial cell cultures, approximately 2% of the total RNA was retained by an oligodeoxythymidylate [oligo(dT)] column.

Poly(A)$^+$ RNAs were translated in a rabbit reticulocyte lysate system (26) for their ability to direct the synthesis of keratins. Figure 1 shows the electrophoretic separation of the translation products before and after immunoprecipitation with anti-keratin antiserum. Rabbit antisera against human stratum corneum keratins (46 to 63 kd) and against the 40-kd keratin were both used to identify the translation products. Keratins translated in vitro from epithelial mRNAs were compared with those obtained from cytoskeletal extracts of the cultured cells.

All of the keratins produced by the epithelial cells in culture (Fig. 1, lanes 1, 5, 9, and 13) were also translated in vitro from the corresponding mRNA preparations (lanes 2, 6, 10, and 14). Similar to cultured human epidermal cells, human epidermal mRNA translated four major keratins (46, 50, 56, and 58 kd) and a minor keratin (52 kd) (Fig. 1, tracks 1 through 4). A two-dimensional gel profile of these translation products revealed the presence of two major isoelectric variants for the 46- and 50-kd acidic keratins and one major isoelectric species for the 56- and 58-kd basic keratins (Fig. 2A). No major additional isoelectric variants were observed for the keratins extracted from whole cells labeled with $^{32}P_i$, although the minor 52-kd keratin and both isoelectric variants for the 50-kd keratin were clearly phosphorylated (data not shown).

The immunoprecipitated translation products of mRNA isolated from the squamous cell carcinoma line were also identical in electrophoretic mobility and immunological properties to the keratins produced in vivo by this cell line (Fig. 1, tracks 5 through 8). Most notably, the acidic 40-kd keratin that is characteristic of the squamous cell carcinomas was clearly translated from its own mRNA (Fig. 2B). This 40-kd translation product could not have been generated from in vitro processing of a larger keratin translation product, since both the SCC-15 and the normal epidermal cells make the same large keratin mRNAs, and yet only the SCC-15 cell mRNA

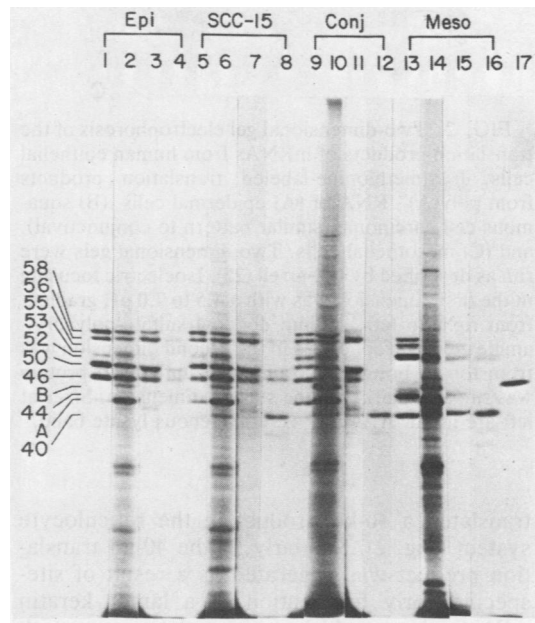

FIG. 1. Identification of mRNAs for the keratins of different human epithelial cells. Keratins and poly(A)$^+$ RNA were extracted from epidermal cells (Epi), squamous cell carcinoma (SCC-15), conjunctival cells (Conj), and mesothelial cells (Meso). The RNAs were translated in vitro, and translation products were precipitated with antiserum. All samples were resolved by electrophoresis through an 8.5% polyacrylamide gel. Lanes 1, 5, 9, and 13, Keratins from [^{35}S]methionine-labeled cellular extracts. Note that actin (A) is a contaminant in these extracts. Lanes 2, 6, 10, and 14, Total [^{35}S]methionine-labeled translation products of poly(A)$^+$ mRNA. Note that the 45-kd band (B) is an mRNA-independent endogenous product from the reticulocyte system (see lane 17). Lanes 3, 7, 11, and 15, Same translation products after precipitation with antiserum to human stratum corneum keratins. Lanes 4, 8, 12, and 16, Translation products after precipitation with antiserum to the SCC-15 cell 40-kd keratin. Sizes at left are in kd.

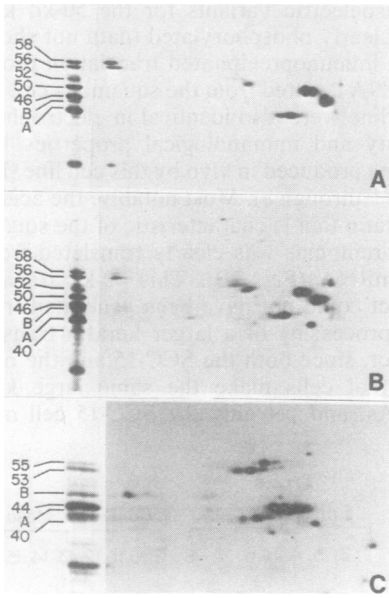

FIG. 2. Two-dimensional gel electrophoresis of the translation products of mRNAs from human epithelial cells. [^{35}S]methionine-labeled translation products from poly(A)$^+$ RNA of (A) epidermal cells, (B) squamous cell carcinoma (similar pattern to conjunctival), and (C) mesothelial cells. Two-dimensional gels were run as described by O'Farrell (25). Isoelectric focusing in the first dimension was with a 4.5 to 7.0 pH gradient from right to left; sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension was from top to bottom. A sample of unfocused protein was run as a marker in the second dimension. Sizes at left are in kd. A, Actin; B, endogenous lysate band.

translated a 40-kd product in the reticulocyte system (Fig. 2). Similarly, if the 40-kd translation product was generated as a result of site-specific early termination of a larger keratin mRNA, this would be expected to occur with equal frequency from normal epidermal cell mRNA. Rather, a distinct 40-kd keratin mRNA seems to be expressed and translated by several cell lines derived from human squamous cell carcinomas, but not by the corresponding cells cultured from nonmalignant tissues.

An mRNA encoding a 40-kd keratin was also detected in cultured conjunctival and mesothelial cells (Fig. 1). Antisera prepared aginst the 40-kd keratin of SCC-15 cells precipitated the translated 40-kd polypeptide from both of these sources (tracks 12 and 16, respectively). In addition, mesothelial cells also produced mRNAs that translated 53-, 55-, and 44-kd keratins. All of these proteins cross-reacted weakly but specifically with antibody specific to human stratum corneum keratins (Fig. 1, lane 15). Similar to those made in vivo, the translated mesothelial keratins differed not only in size but also in degree of microheterogeneity and in isoelectric mobility from the epidermal keratins (Fig. 2C). Thus, even though these keratins are related to the epidermal keratins both antigenically and as judged by their one-dimensional peptide (46), they are clearly translated from their own mRNAs.

Hybridization of epithelial keratin mRNAs with cloned epidermal keratin cDNAs. The nearly full-length cloned keratin cDNAs used in this study were constructed from the mRNAs of cultured human epidermal cells and are described elsewhere (12). In a positive hybridization-translation assay, one cloned cDNA (KA-1) selected from a total population of epidermal mRNAs those encoding the 56- and 58-kd keratins, whereas the other cloned cDNA (KB-2) selected those encoding the 46- and 50-kd keratins. To determine whether the keratin mRNAs of other human epithelial cells share sequence homologies with one or both of these cDNAs, we conducted positive hybridization-translation assays (5, 31). The translation products of epithelial cell mRNAs that hybridized specifically to either of the two cloned keratin cDNAs are shown in Fig. 3. The products were identified as keratins by their sizes, isoelectric points, specific precipitation (by antiserum against either human stratum corneum keratins or the 40-kd keratin), and one-dimensional polypeptide patterns generated by *Staphylococcus aureus* V8 protease (12, 13, 15).

All of the keratin mRNAs from all epithelial cell types hybridized to one or the other of the two classes of cloned keratin sequences. Under standard conditions of stringency, no mRNA hybridized to both classes. Hybridization was dependent on the size of the corresponding keratin encoded by the mRNA. The mRNAs encoding the 58- and 56-kd keratins of normal epidermis, squamous cell carcinoma, and conjunctiva all hybridized, although to various extents, with KA-1, the cloned 56- to 58-kd epidermal keratin cDNA (Fig. 3, lanes 2, 6, and 10). The mRNAs encoding the 53- and 55-kd keratins of mesothelial cells (lane 14) and a minor 55-kd keratin of conjunctival cells (lane 10) also hybridized with KA-1. In contrast, the mRNAs encoding the smaller keratins (40- to 52-kd) all hybridized with KB-2, the cloned 46- to 50-kd epidermal keratin cDNA. mRNAs encoding the following keratins were specifically selected by this cloned cDNA: (i) the 46- and 50-kd keratins of normal epidermal cells (lane 3); (ii) the 40-, 46-, 50-, and 52-kd keratins of squamous cell carcinoma cells and a minor (as yet uncharacterized) 35-kd protein (lane 7); (iii) the 40-, 46-, 50-, and 52-kd keratins of conjunctival cells (lane 11); and (iv) the 40- and 44-kd keratins of mesothelial

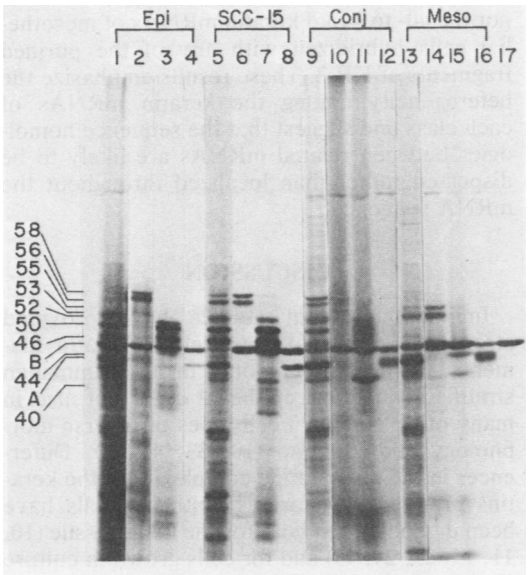

FIG. 3. Identification of two distinct classes of keratin mRNAs expressed in different human epithelial cells. Poly(A)$^+$ RNA was isolated from epidermal cells (Epi, lanes 1 through 4), squamous cell carcinomas (SCC-15, lanes 5 through 8), conjunctival cells (Conj, lanes 9 through 12), and mesothelial cells (Meso, lanes 13 through 16). Lanes 1, 5, 9, and 13, Translation products of unfractionated poly(A)$^+$ RNA. Lanes 2, 6, 10, and 14, Translation products of mRNA that specifically hybridized to DNA from pKA-1, a hybrid plasmid containing a 56-kd epidermal keratin cDNA insert. Lanes 3, 7, 11, and 15, Translation products of mRNA that specifically hybridized to DNA from pKB-2, a hybrid plasmid containing a 50-kd epidermal keratin cDNA insert. Lanes 4, 8, 12, and 16, Translation products of mRNA that specifically hybridized to plasmid DNA from pA-1, a hybrid plasmid containing human actin cDNA. Lane 17, An mRNA-independent artifact of the reticulocyte lysate system. Sizes at the left are in kd. A, Actin; B, mRNA-independent artifact (a minor band at 150 kd and several smaller-size bands were also artifacts of the translation system).

cells (lane 15). In control reactions, a cloned human epidermal actin cDNA, A-1 (E. Fuchs, K.-H. Kim, I. Hanukoglu, and N. Tanese, *in* H. Ogawa and I. Bernstein, ed., *Symposium on Normal and Abnormal Keratinization*, in press), selected from each preparation of epithelial cell mRNA that fraction of mRNAs encoding actin, a cytoskeletal component common to all epithelia (Fig. 3, lanes 4, 8, 12, and 16).

Discrimination between related keratin mRNA sequences. To evaluate the extent of homology within each of the two groups of epithelial keratin mRNAs, we varied the temperature of mRNA elution from the cDNA hybrids. Positive hybridization-translation assays were repeated, but this time specifically bound mRNA was eluted from the nitrocellulose filters under progressively higher temperatures. The translation products of the eluted mRNAs were separated electrophoretically (Fig. 4).

When total mRNA from human squamous cell carcinoma (SCC-15) cells was hybridized at 41°C with filter-bound KA-1 cDNA, the 56- and 58-kd keratin mRNAs were specifically selected. When eluted under conditions of low stringency (65°C), 75% of the mRNA encoding the 58-kd keratin was removed from the filter, along with only 5% of the 56-kd keratin mRNA (Fig. 4, lane 2). When the temperature was increased to 85°C (lane 3), the remainder of the 58-kd keratin mRNA was removed, along with 95% of the 56-kd keratin mRNA. Similar results were obtained with the mRNAs from both normal epidermal and conjunctival cells, suggesting that the se-

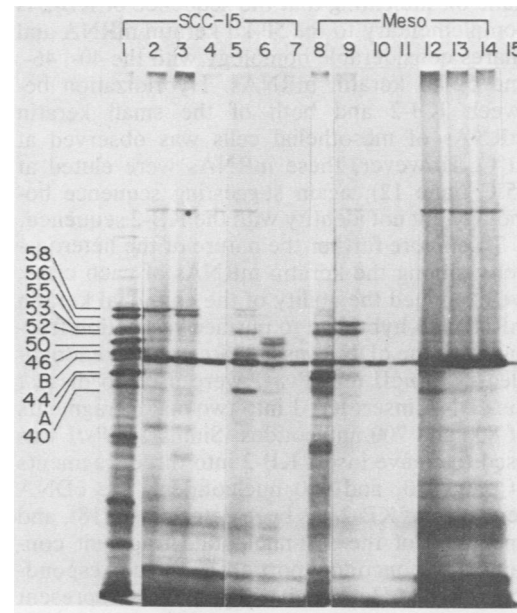

FIG. 4. Temperature-dependent discrimination between related keratin mRNA sequences and the two classes of cloned epidermal keratin cDNAs. Lanes: 1 and 8, translation products of unfractionated poly(A)$^+$ RNA; 2 through 4 and 9 through 11, translation products of mRNAs that specifically hybridized to KA-1 (56-kd keratin) cDNA and then eluted at 65, 85, and 100°C, respectively; 5 through 7 and 12 through 14, translation products of mRNA that specifically hybridized to KB-2 (50-kd keratin) cDNA and then eluted at 65, 85, and 100°C, respectively; 15, translation product of the reticulocyte lysate without added mRNA. Sizes shown at the left are in kd. A, actin; B, mRNA-independent artifact (a minor band at 150 kd was also an artifact of the system). The poly(A)$^+$ RNA in lanes 1 through 7 was isolated from human epidermal cells and in lanes 8 through 14 from mesothelial cells.

quence of KA-1 is complementary to the mRNA for the 56-kd keratin and shares significant homology with the mRNA for the 58-kd keratin. When total mRNA from human mesothelial cells was hybridized at 41°C with KA-1 cDNA, both the 53- and the 55-kd keratin mRNAs were selected. However, these mRNAs were removed under conditions of low stringency (65°C, lane 9), indicating sequence homology but not identity with the KA-1 insert.

In similar assays with KB-2, the 40-, 46-, 50-, and 52-kd keratin mRNAs of SCC-15 cells hybridized strongly at 41°C, with weak hybridization of the 56- and 58-kd keratin mRNAs. Under conditions of low stringency (65°C), the mRNAs for the 52-, 56-, and 58-kd keratins were completely removed, along with 90% of the 40-kd and 50% of the 46-kd keratin mRNAs (Fig. 4, lane 5). Almost no 50-kd keratin mRNA was eluted until the temperature was raised to 85°C (lane 6), indicating that the sequence of KB-2 is complementary to the 50-kd keratin mRNA and shares considerable homology with the 40-, 46-, and 52-kd keratin mRNAs. Hybridization between KB-2 and both of the small keratin mRNAs of mesothelial cells was observed at 41°C. However, these mRNAs were eluted at 65°C (lane 12), again suggesting sequence homology but not identity with the KB-2 sequence.

To explore further the nature of the heterogeneity among the keratin mRNAs of each class, we examined the ability of the epithelial keratin mRNAs to hybridize to purified restriction fragments of the cDNA inserts. Restriction endonucleases *Hin*cII and *Pvu*II were used to dissect the cDNA insert KA-1 into two major fragments of 800 and 700 nucleotides. Similarly, *Pst*I was used to cleave insert KB-2 into three fragments of 570, 470, and 380 nucleotides. The cDNA sequence of KB-2 has been determined (18), and only 20% of the 570-nucleotide fragment contains any noncoding portion of the corresponding mRNA. Hence, these fragments represent mostly the coding portions of the mRNA sequence. We purified these fragments and tested their ability to hybridize under stringent conditions with the keratin mRNAs of SCC-15 and mesothelial cells (data not shown).

In both cases, the hybridization patterns for the insert fragments were indistinguishable, although fewer mRNAs hybridized with the fragments than with the intact insert. Thus, the two fragments of KA-1 hybridized only with the 56-kd SCC-15 cell keratin mRNA, whereas the intact KA-1 hybridized with both the 56- and 58-kd keratin mRNAs. Similarly, the three fragments of KB-2 hybridized with only two (46 and 50 kd) of the four SCC-15 cell keratin mRNAs that hybridized with the intact insert. Neither the 40- to 52-kd keratin mRNAs of SCC-15 cells nor the 40- to 44-kd keratin mRNAs of mesothelial cells hybridized with any of the purified fragments of KB-2. These results emphasize the heterogeneity among the keratin mRNAs of each class and suggest that the sequence homologies between related mRNAs are likely to be dispersed rather than localized throughout the mRNA sequence.

DISCUSSION

Immunofluorescent studies have provided strong evidence that cytoskeletal keratin filaments are present not only in all mammalian stratified squamous epithelial cells, but also in many other epithelial cell types of diverse morphology and function (10, 33, 41–43). Differences in the polypeptide complexity of the keratins produced by various epithelial cells have been demonstrated both for the intact tissue (10, 11, 15, 23, 24, 43) and for cells grown in culture (9, 16, 40, 41, 46). However, it was not determined previously whether this variation in keratins with cell type represents tissue-specific differences in the synthesis of keratin mRNAs or, alternatively, posttranslational processing or modification of a defined set of keratin polypeptides.

In this paper, we have shown conclusively that the different keratins synthesized by different epithelial cells originate from two distinct classes of mRNA sequences whose members are homologous but not identical. In earlier studies, we showed that the multiple epidermal keratin mRNAs can be divided into two different groups, as judged by their ability to hybridize to one of two separate classes of cloned epidermal keratin cDNA sequences (12). We have now shown that all of the keratin mRNAs from cells cultured from other stratified squamous epithelia and from simple squamous epithelia fall into these same two classes. mRNAs that code for keratins of 53 to 58 kd belong to one sequence class, and mRNAs that code for keratins of 40 to 52 kd belong to the other sequence class. For every epithelial cell type thus far investigated, at least one member of each of the two classes is always expressed. This is true even for mesothelial cells, which produce a subset of keratins totally different from any of the major keratins made by epidermal cells.

Of particular interest is our finding that the 40-kd keratin present in several squamous cell carcinoma lines from malignant epidermis and oral epithelium is synthesized from its own mRNA. In normal epidermis and in cultured epidermal cells, this protein is only synthesized when the level of vitamin A is 10-fold higher than the physiological level (16). Its presence in the malignant cell lines grown with normal con-

centrations of vitamin A is clearly due to the synthesis of a new keratin mRNA that seems to be related in sequence to the mRNAs encoding the 46- and 50-kd keratins of normal epidermal cells. Whether the 40-kd keratin mRNA arises from an aberrant splicing of the transcript for the 46- to 50-kd keratin genes or, alternatively, from abnormal expression of a separate gene for the 40-kd keratin remains to be determined.

Previously we showed that the two distinct classes of keratin mRNA sequences are each encoded by a subfamily of about 10 genes per haploid human genome (12). These two subfamilies of keratin genes are coordinately conserved throughout vertebrate evolution, raising the intriguing possibility that both classes of keratins may be required for filament assembly. The structural importance of more than one keratin sequence was initially postulated by Steinert et al. (37) and Lee and Baden (21), who observed that the assembly of keratin filaments in vitro requires at least two different purified keratin polypeptides. Our finding that members of two distinct sequence classes of keratin mRNAs are present in cells cultured not only from epidermis but also from a variety of diverse and morphologically distinct epithelial tissues provides strong support for their functional significance.

We do not know yet whether all of the keratin genes within each subfamily are distinct and whether they are all expressed. In other multigene families, e.g., those encoding the actins and the tubulins, some of the genomic sequences have been shown to contain genetic lesions, including deletions, insertions, and in-frame termination codons, rendering them incapable of being expressed (8, 44). In addition, even though we showed that the mRNAs for the differentially expressed keratins are distinct, our data do not strictly rule out the possibility that multiple modes of processing a single heteronuclear transcript might be responsible for generating the diversity within each class. However, if variation in posttranscriptional processing were the sole source of the heterogeneity, then some regions of mRNA sequence should be common to several, if not all, members within each class, whereas other stretches of sequence should be unique to a particular mRNA. Hence, some restriction endonuclease fragments of a cloned keratin cDNA of one class might be expected to hybridize uniformly with all epithelial mRNAs of that class, whereas other fragments would be unique to a single mRNA and would hybridize selectively even under less stringent conditions. Our finding of little or no variability in the pattern of mRNAs that hybridized to the partial sequences of either insert suggests that the sequence differences within a class of keratin mRNAs are probably dispersed rather than localized. Thus, it seems most plausible that the sequence differences within a class of keratin mRNAs reflect a series of gene duplications, followed by divergence, to create a subfamily of similar but not identical genes.

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