The Nature and Significance of Differential Keratin Gene Expression[a]

ELAINE FUCHS, ISRAEL HANUKOGLU, DOUGLAS MARCHUK, MICHAEL P. GRACE, AND KWAN HEE KIM

The Department of Molecular Genetics and Cell Biology
The University of Chicago
Chicago, Illinois 60637

Keratin filaments are 8 nm in diameter, and together with the 6 nm actin microfilaments and the 23 nm microtubules, they constitute the cytoskeletal architecture of most vertebrate epithelial cells.[1,2] The filaments are assembled from two classes of subunits, type I and type II.[12,13–16] The type I keratins are acidic and generally small in size (40–57 kD). The type II keratins are more basic and larger in size (53–67 kD).[11,12] Each class is encoded by a small multigene family,[12,15] the members of which are differentially expressed in different epithelial cells,[10,11,17–20] at different stages of differentiation and development,[6,17,21–25] and upon malignant transformation.[26–28] At any given time, an epithelial cell typically expresses two to five keratin polypeptides, including at least one member of each type.[12,15,20,29] Prior to the discovery of the two distinct types of keratin subunits and their coordinate expression, it was shown that no single keratin protein of either type by itself was competent for filament assembly.[30–32] Collectively, these results suggest that the expression of both types may be essential for subsequent filament formation.

The details of how the keratin subunits assemble remain to be elucidated. Until recently, most of what we knew about the structure of the keratin filament came from the early X-ray diffraction studies of Crick and Pauling back in the early 1950s.[33,34] The 8 nm keratin filament was known to be made up of an as yet undetermined number of smaller, closely packed filaments, called protofilaments. Each protofilament has a diameter of about 2 nm and is itself made up of several α-helical polypeptide strands intertwined in a coiled-coil configuration.

The early X-ray studies could not decipher whether two or three helical polypeptide subunits were interwoven to give the coiled-coil substructure of the protofilament. Although physicochemical studies suggested a three-stranded coiled coil,[35,36] model

[a]E. F. is a Searle Scholar, the recipient of a National Science Foundation Presidential Young Investigator Award, and a National Institutes of Health Career Development Award. K. H. K. is a Postdoctoral Fellow funded by the National Cancer Institute. M. P. G. is a Postdoctoral Fellow funded by the Damon Runyon-Walter Winchell Cancer Fund. D. M. is a National Institutes of Health Predoctoral Trainee. This work was supported by a grant from the National Institutes of Health.

building using helical portions of a wool keratin showed that the sequence is compatible with a two-stranded, but not a three-stranded coiled coil.[37] Additional evidence from chemical cross-linking analyses also favors a protofilament consisting of a pair of fibrous polypeptides interwoven in a coiled-coil structure.[38-40] More recent electron microscopy studies suggest that several higher order coiled coiling of protofilaments may take place during the assembly of the 8 nm filament.[41] Although the controversies over the substructure of the keratin filament have not yet been unequivocally resolved, it seems likely that a type I and a type II keratins are both involved in the formation of the basic subunit structure of the protofilament.

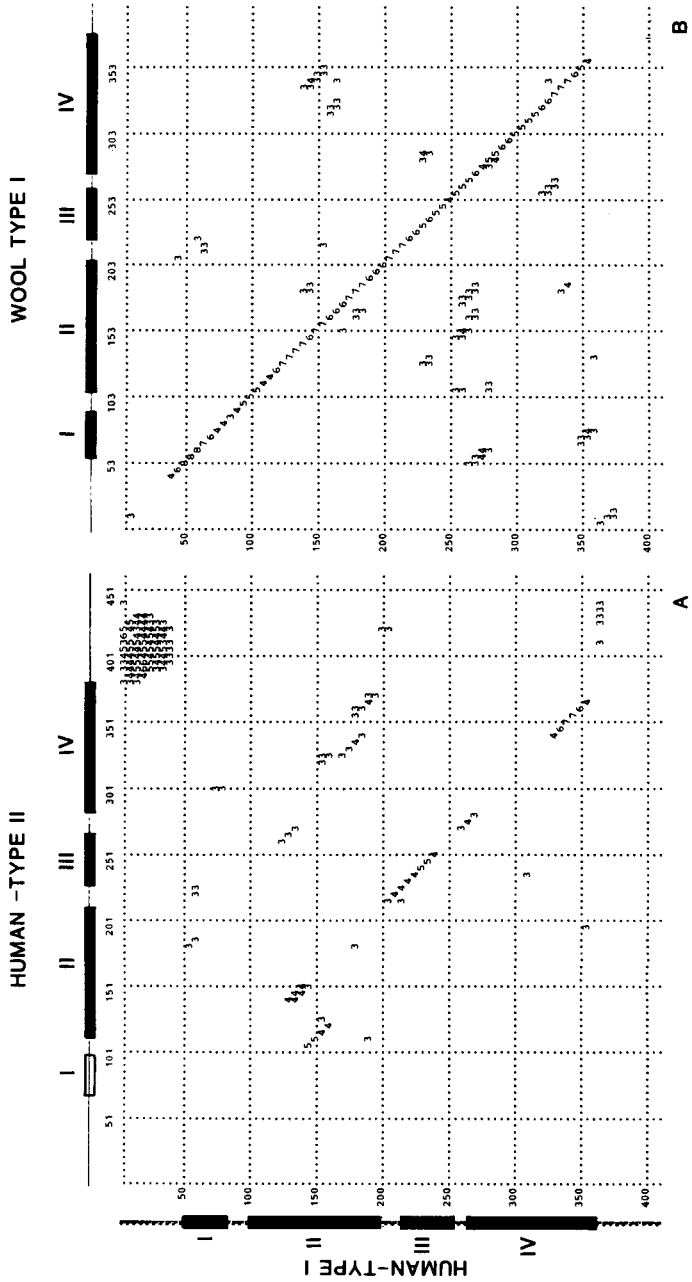
Four major questions must now be considered: (1) What are the details of the structural interactions between a pair of type I and type II keratin subunits that lead to the formation of the protofilament? (2) What is the nature of the differences between the individual members of the type I or type II family and how do these differences influence the resulting structure or function of the 8 nm filaments expressed in different epithelial tissues? (3) What is the relation between the keratins and other IF subunits? (4) What is the complexity of the genes encoding the different keratins and how is their expression regulated? In this paper, we discuss work done in our own laboratory and the laboratory of others where techniques in molecular biology have been utilized to begin to unravel the answers to these questions.

THE AMINO ACID SEQUENCES FOR A TYPE I AND TYPE II KERATIN EXPRESSED IN HUMAN EPIDERMAL CELLS AND THEIR PREDICTED SECONDARY STRUCTURES

To explore whether a type I and type II keratin might interact to form the coiled coil of the protofilament, it is necessary to determine sequences of a pair of type I and type II keratins that are co-expressed in a particular epithelial tissue. We have obtained the predicted amino acid sequences for the 50 kD type I and the 56 kD type II keratin co-expressed in cultured human epidermal cells. These sequences were determined in large part from cloning and sequencing near full-length cDNAs complementary to their mRNAs.[16,42] We have recently completed the amino acid sequence of the type I keratin by isolating and sequencing the gene encoding the human 50 kD keratin.

A comparison of the predicted amino acid sequences for the two keratins reveals that they are only very distantly related to one another with $< 30\%$ homology (FIGURE 1, A). The strongest sequence homology resides in residues 200-350. A glycine-serine-rich sequence in the amino terminal region of the type I 50 kD keratin (residues 1-50) shows weak homology with a similar sequence in the carboxy terminal region of the type II 56 kD keratin (residues 380-440).

A remarkable feature of the two keratins is that even though they have very different sequences, their predicted secondary structures are largely indistinguishable (FIGURE 2).[16] Computer-assisted analyses of these sequences have shown that a central, region (approximately 300 amino acid residues long) of both the type I and the type II keratins consists of four richly α-helical domains (noted by thick bars) demarcated from one another by three regions for which β-turns or helix breaking is predicted with a high degree of probability. These helical domains are nearly equal in length for the two keratins, even though the type II keratin is always substantially larger than the co-expressed type I keratin. The difference in size of the keratins seems to


HUMAN -TYPE II
WOOL TYPE I
I
II
III
IV
A
B
HUMAN-TYPE I
I
II
III
IV

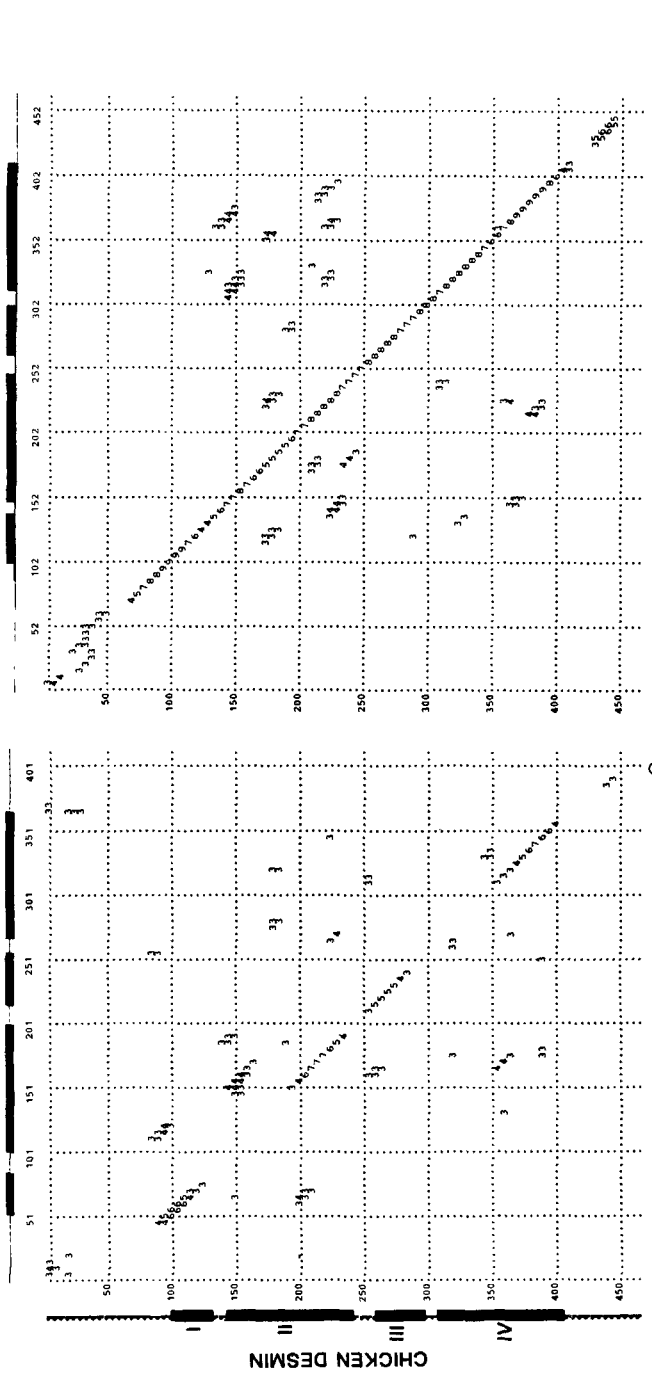


FIGURE 1. Matrix plot of homology between (A) a human type I (50 kD) and a human type II (56 kD) epidermal cytoskeletal keratin; (B) the human type I and a wool microfibrillar type I keratin; (C) chicken desmin and the human type I keratin; and (D) chicken desmin and hamster vimentin. The numbers on the axes correspond to the residue numbers of the respective sequences. The sequences were obtained from the following sources: human type I,[42] human type II,[16] wool type I,[43] chicken desmin,[40] and hamster vimentin.[44] The positions of the four predicted helical domains (see FIGURES 2 and 3) are marked by bars (I–IV) along the residue axes. Note that the sequences of the amino terminal end and the first predicted helical region (I) of the human type II keratin are not yet known, although their structures are expected to be similar to those of the human type I keratin.[16] The matrix plot analysis was carried out by a modified version of Pustell and Kafatos (1982) program. The parameters used in the analysis: range = 20, distance weight factor = 1, compression factor = 4, cutoff point = 34%. Thus, the position of each number on the plots indicates that within a 20 residue long segment to the right of the marked position, at least 35% of the residues are identical in both sequences. The numbers indicate the percentile range of identical residues in the 20 residue stretch, 3 = 35-39%, 4 = 40-49%, . . . 9 = 90-99%.

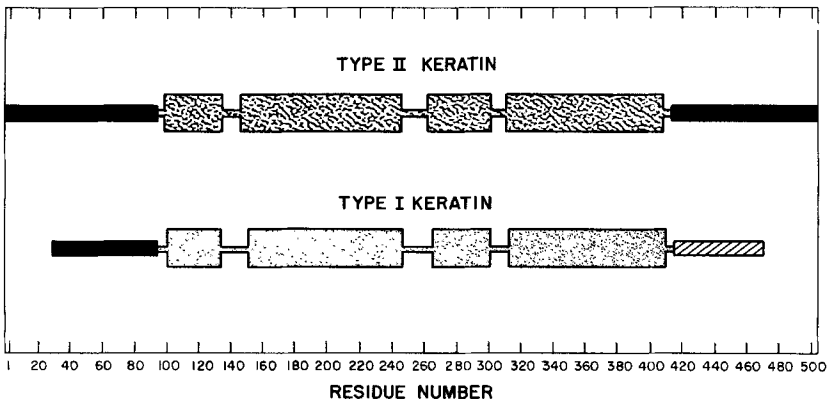


FIGURE 2. Comparison of the predicted secondary structures of the 56 kD type II and 50 kD type I keratins that are coordinately expressed in cultured human epidermal cells. The predicted amino acid sequences of the two keratins and their predicted secondary structures were determined previously.[16,42] The values on the abscissa indicate amino acid residue number. The sequences have been aligned according to their predicted secondary structures. The Chou and Fasman[45,46] and Garnier, Osguthorpe and Robson[47] methods used in our analyses make predictions for four conformational states: α-helix, β-sheet, β-turn, and random coil. For each keratin, central α-helical domains are represented by the thick bars. The thinner internal bars mark the positions of the β-turns, or helix breakers, that are predicted by the Chou and Fasman method for all IF protein sequences. The bars flanking the helical region represent the non-helical termini. Largely different sequence homologies are indicated by different shades of gray.

reside solely in their nonhelical amino and carboxy termini, which are highly variable in length. These general structural features of the pair of human epidermal keratins are also very similar to those of the pair of partially sequenced wool keratins,[43,62] and they are likely to represent structural characteristics common to all keratins.

The central α-helical domains of the keratin subunits are likely to be involved in the coiled coil of the 2 nm protofilament. The sequence characteristic of a periodic interchain interaction, i.e., a heptad repeat of **a b c d e f g**, where **a** and **d** are hydrophobic residues, has been identified in the helical portions of all sequenced keratins.[16,42,48,49] In addition, an interesting periodicity in positively and negatively charged residues has been observed for different keratin subunits, indicating that electrostatic interactions may also play an important role in stabilizing the keratin structure.[50,51]

The overall structural features of the type I and type II keratins support the conservation and coordinate expression data that implicate these two types of sequences in forming the structural backbone of the keratin filament. These data do not necessarily rule out the possibility that two members of a single keratin type could form a keratin filament. However, coupled with the finding that no single keratin can self-assemble into a filament[30–32] and with recent assembly data that suggest that filaments from particular mixtures of type I and type II keratin subunits form with different stabilities,[52] it seems likely that the two subunits are intimately involved in the coiled coil of the protofilament. This apparent requirement for two subunits in filament formation seems to set the keratins apart from other intermediate filament subunits, which have structural features similar to the keratins, but which can assemble as homopolymers.[40,53–61]

THE SEQUENCE VARIATIONS AMONG DIFFERENT MEMBERS OF A SINGLE KERATIN TYPE AND THEIR FUNCTIONAL SIGNIFICANCE

Although the type I and type II keratin subunits are only distantly evolutionarily related, hybridization studies indicate that the individual members within a particular class are quite similar to each other.[15,20] The nature of these more subtle differences is important, since it is the members of each class that are differentially expressed in different tissues. Sequence analyses of all the different type I and type II keratins should resolve whether the multiplicity of keratin sequences is simply an evolutionary phenomenon, or whether the use of different keratin subunits in filament assembly influences the properties, structure, or interactions of the resulting filaments.

To date sequence data for the keratins are limited. In addition to the sequences of the two human keratins that we have obtained,[16,42] there is complete sequence data for a type I keratin of mouse[48] and partial sequence data for a type I and a type II keratin of wool.[43,62] Nevertheless, from a comparison of these few sequences, an intriguing relation is emerging. For any two keratins of the same type, the α-helical domains of the polypeptides are quite highly conserved (FIGURE 1B and FIGURE 3). This is in direct contrast to a comparison of two keratins of different types, where these domains are largely distinct (FIGURE 1A and FIGURE 2). The major differences in the sequences of different subunits of the same type reside almost exclusively in the nonhelical termini of the keratins.[16,42,48,62] These differences are most pronounced when the keratins of wool are compared with the keratins of epidermis (FIGURE 1, B). Whereas the end segments of the epidermal keratins contain a strange inexact

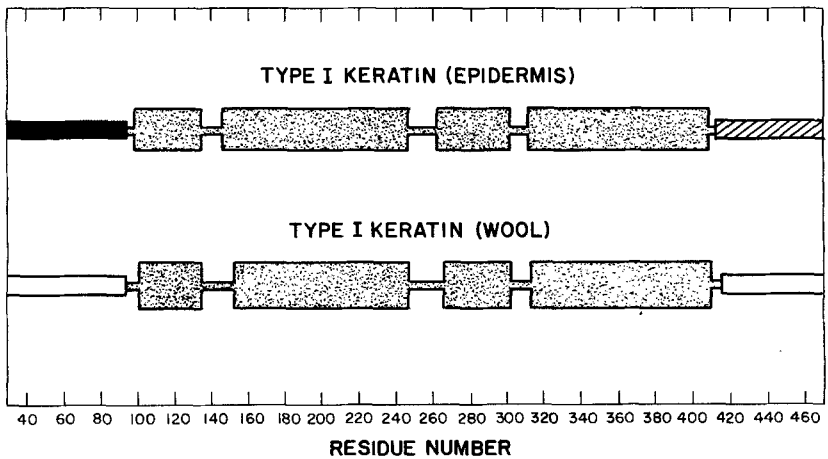


FIGURE 3. Comparison of predicted secondary structures of the 50 kD epidermal type I and the wool type I keratins that are differentially expressed in different tissues. The predicted secondary structures were determined previously.[16,42,62] The values on the abscissa indicate amino acid residue number. The sequences have been aligned according to their predicted secondary structures, which were determined as indicated in the legend to FIGURE 1. The thick bars represent α-helical domains, the thinner internal bars mark positions where the helix is broken, and the bars flanking the central helical region represent the non-helical termini. Largely different sequence homologies are indicated by different shades of gray.

repeat consisting of glycine and serine residues interspersed with phenylalanine, tyrosine, and leucine, the wool keratins have termini that are conspicuously rich in cysteine (TABLE 1). Such differences are quite remarkable when it is considered that the central helical domains of the two type I or type II keratins are highly homologous. Thus, the keratins of a particular class, either type I or type II, seem to have constant helical domains and variable nonhelical termini.

The functional significance of the multiplicity of keratin sequences and their differential expression still remains largely undetermined. However, a closer look at the nonhelical termini and the role that these termini play in filament assembly has shed some light on this issue. Elegant physicochemical studies by Skerrow, Matoltsy, and Matoltsy,[35] and also by Steinert and colleagues[36] have revealed that the removal of these ends by limited proteolytic digestion render the subunits incompetent for filament assembly. The subunits intertwine to form the 2 nm coiled coil of the protofilament, but no protofibrillar interactions take place to yield the 8 nm diameter of the filament. The end-to-end interactions of the subunits are also disrupted, leaving rods that are 0.05 μm rather than 40 μm in length. Thus, the use of subunits with different termini in filament assembly might not necessarily change the structure of the resulting 8 nm filaments, but it is likely to give rise to filaments with quite different properties. The microfibrillar keratin filaments are insoluble and possibly rigid, with numerous disulfide bridges linking the termini of the subunits to one another, whereas the epidermal cytoskeletal keratin filament is probably more flexible with glycine-serine-rich termini and relatively few cysteine residues.[16,42,48,62] Although the variations among the individual keratin subunits of each class are not likely to be more pronounced than those already seen between the keratins of wool and epidermis, it is expected that smaller variations in the sequences of other epithelial keratins will give rise to subtle variations in the properties of the corresponding filaments, e.g. their solubility, their flexibility, their tensile strength, and their ability to interact with the cell or nuclear membrane or other cytoplasmic components.

A COMPARISON OF TYPE I AND TYPE II KERATIN WITH OTHER IF SUBUNITS

Sequence analysis of different IF subunits has revealed that all IF proteins are evolutionarily related.[13,14,16,40–44,48,55–58,61,62] A summary of the IF sequence data suggests that if all IF proteins originated from a single gene, then two distant duplications must have led to the formation of a third IF gene in addition to the primordial type I and type II keratin genes.[64] Each of these genes must have undergone further, more recent, duplication to give rise to three related multigene subfamilies. The third gene subfamily is made up of the genes encoding the neurofilament proteins, glial filament proteins, desmin, and vimentin, all of which share 60-70% homology with one another.[65] This IF multigene subfamily shows 25-40% homology with both the type I and type II keratin subfamilies. FIGURE 1 illustrates the low but substantial homology between desmin and the 50 kD human type I keratin (1C) and the much higher homology between desmin and vimentin (1D).

For all IF subunits, the predicted secondary structures are strikingly similar.[16,40,42,48,62,65] In comparing any two members of the same subfamily, the central α-helical domains share marked homology, whereas the variation in sequence resides primarily at the nonhelical termini (TABLE 1).

TABLE 1. The Amino Acid Compositions of the Different Structural Domains of Epidermal (Cytoskeletal) and Wool (Microfibrillar) Keratins and Intermediate Filament Proteins Desmin and Vimentin

	Amino Terminus						Central Region				Central Region			Carboxy Terminus						
	WT-I	MT-I	HT-I	WT-II[a]	CDES	HVIM	WT-I	MT-I	HT-I	HT-II[a]	WT-II[a]	CDES	HVIM	WT-I[a]	MT-I	HT-I	HT-II	WT-II[a]	CDES	HVIM
Ala	3.7	0.0	5.2	5.5	6.1	4.0	4.8	6.3	8.0	10.0	8.4	10.7	8.4	12.1	0.9	0.0	2.3	6.3	5.4	0.0
Arg(+)	5.6	2.8	3.5	5.5	11.2	13.0	9.0	7.0	7.7	7.1	9.7	9.4	8.7	6.1	6.2	8.7	2.3	4.8	5.4	5.5
Asn	11.1	0.7	0.9		2.0	5.0	7.1	6.7	6.1	5.9	4.6	4.9	5.2	0.0	0.0	2.2	2.3		1.8	7.3
Asp(−)	1.9	0.7	0.9	(7.3)	2.0	3.0	4.8	4.8	6.4	8.2	5.1	5.8	7.1	0.0	0.9	6.5	0.0	(6.3)	3.6	7.3
Cys	14.8	2.1	2.6	6.4	0.0	0.0	2.6	0.6	0.6	0.7	3.0	0.3	0.3	18.2	0.9	0.0	0.0	17.5	0.0	0.0
Gln	0.0	0.7	0.9		6.1	2.0	8.7	8.6	6.4	7.1	3.8	8.4	9.7	0.0	0.9	8.7	2.3		8.9	3.6
Glu(−)	1.9	0.0	0.9	(12.8)	2.0	1.0	15.4	13.3	14.1	11.9	16.5	15.2	15.9	0.0	0.0	2.2	1.1	(3.2)	10.7	9.1
Gly	9.3	49.6	35.0	9.2	9.2	7.0	1.0	2.9	2.9	3.0	2.5	2.3	1.9	6.1	58.4	4.3	29.5	7.9	3.6	1.8
His	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.3	0.6	1.1	0.8	1.6	1.0	0.0	0.0	4.3	1.1	0.0	5.4	5.5
Ile	1.9	0.0	2.6	4.6	0.0	1.0	4.2	4.8	4.5	4.8	6.3	5.5	3.9	3.0	0.0	2.2	3.4	0.0	5.4	3.6
Leu	5.6	3.5	6.0	11.9	6.1	7.0	14.7	14.6	12.8	12.3	9.7	11.7	12.6	3.0	0.0	2.2	4.5	7.9	3.6	18.2
Lys(+)	0.0	0.7	1.7	5.5	0.0	1.0	3.5	5.4	6.1	7.4	5.9	6.1	6.1	3.0	0.9	6.5	3.4	0.0	5.4	3.6
Met	0.0	0.0	1.7	0.9	1.0	2.0	0.3	1.9	3.8	2.6	1.7	2.9	2.6	0.0	0.0	2.2	0.0	0.0	3.6	0.0
Phe	7.4	9.9	6.0	4.6	6.1	3.0	1.0	0.6	1.3	1.5	1.3	1.6	1.9	0.0	0.9	2.2	1.1	0.0	3.6	1.8
Pro	9.3	0.0	0.9	5.5	4.1	4.0	1.0	1.0	1.0	0.4	0.4	0.6	0.3	18.2	0.9	0.0	0.0	4.8	3.6	5.5
Ser	18.5	22.7	21.6	7.3	19.4	25.0	7.4	7.6	4.5	4.1	5.9	2.3	4.2	12.1	22.1	26.1	26.1	17.5	8.9	10.9
Thr	3.7	0.7	3.5	4.6	12.2	11.0	4.5	5.4	4.5	3.7	3.4	2.9	2.3	9.1	0.9	8.7	5.7	6.3	12.5	10.9
Trp	1.9	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.6	0.4	0.4	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tyr	0.0	3.5	3.5	3.7	5.1	5.0	2.9	3.2	3.2	3.3	3.8	2.9	2.6	3.0	6.2	0.0	5.7	0.0	0.0	0.0
Val	3.7	2.1	2.6	4.6	7.1	6.0	6.4	4.8	4.8	4.5	6.8	4.5	4.9	6.1	0.0	13.0	9.1	17.5	8.9	5.5

The values are presented as percentages and are based on the sequences from the following sources: (1) WT-I: Type I microfibrillar keratin from sheep wool[13,14,43,62]; (2) MT-I: Type I cytoskeletal keratin from mouse epidermis (M_r = 59K)[48]; (3) *HT-I: Type I cytoskeletal keratin from human epidermal cells* (M_r = 51 K)[42,63]; (4) *HT-II: Type* II cytoskeletal keratin from human epidermal cells (M_r = 56 K)[16]; (5) WT-II: Type II microfibrillar keratin from sheep wool[13,14,43,62]; (6) CDES: Desmin from chicken muscle (M_r = 53 K)[40]; (7) HVIM: Vimentin from hamster (M_r = 53 K).[44]

The "Central Region" represents the segment extending from the first amino acid of Helical Domain I to the last amino acid of Helical Domain IV as previously described.[16]

[a] The composition is based on incomplete sequence for this region.

THE REGULATION OF THE DIFFERENTIAL EXPRESSION OF HUMAN KERATINS IN DIFFERENT TISSUES AND IN MALIGNANT NEOPLASMS DERIVED FROM NORMAL EPITHELIA

It is interesting that the nonhelical termini of the two keratins expressed in human epidermal cells are rich in glycine and serine residues, whereas the two keratins produced by wool are rich in cysteine and serine residues. This suggests that the expression of different pairs of type I and type II keratins may be coordinately regulated. An investigation of the keratins expressed in different tissues suggests that indeed this may be the case.[11] It has been well documented that the expression of most keratins arises from the appearance of newly synthesized mRNAs in these cells.[66,70] Whether the coordinate expression of all of these proteins extends to the level of the genome has not yet been determined, although there are clearly multiple genes (≥ 10) for each of the two keratin subfamilies.[12,15] We have begun to isolate and characterize the multiple human genes encoding the keratins and have shown that they are different, albeit related. In at least some cases, the genes encoding the keratins are differentially expressed. However, we have not ruled out the possibility of additional regulation at the post-transcriptional level.

To further probe the regulation of keratin gene expression, we have chosen differentiating human epidermis as a model system. Previously, we had found that as a human basal epidermal cell is triggered to terminally differentiate, it changes the pattern of keratins that it makes.[17] RNAs encoding keratins of 46, 50, 56, and 58 kD are made by the basal cells, and additional mRNAs encoding keratins of 56.5 and 67 kD are produced by the differentiating keratinocytes (FIGURE 4). The 46 and 50 kD keratins are acidic and belong to the type I class, whereas the 56 and 58 kD keratins are basic and belong to the type II class.[15] We have recently shown that the newly synthesized 56.5 kD keratin is an acidic type I keratin and the 67 kD keratin is a basic type II keratin.[71] We have confirmed this by two-dimensional gel electrophoresis, by immunocross-reactivity and by Northern blot analysis. The shift towards the synthesis of type I and type II keratins of unusually large size occurs in all vertebrate epidermis[12] and seems to occur without an imbalance in the normal ratio of type I to type II keratins within each cell.

We now know that the shift to the synthesis of large keratins is regulated in a negative fashion by the fat-soluble isoprenoid, vitamin A. When human epidermal cells are cultured *in vitro* in the absence of vitamin A, an induction in the synthesis of these large keratins and their mRNAs is observed.[72] Thus, whether directly or indirectly, the vitamin appears to regulate coordinately the expression of at least some pairs of keratins. In addition to the influence of vitamin A on epithelial growth and differentiation, a number of other environmental and extracellular roles have been shown to play a role in gene regulation in these cells. These include dermal-epidermal interactions,[73–75] cAMP effectors,[76] steroid hormones,[77] and growth factors.[78]

That extracellular factors play a prominent role in keratin gene expression can be illustrated by investigating the altered pattern of keratins produced under different conditions in human esophageal tissue (FIGURE 5).[11,79,80] Normal esophageal tissue produces three major keratins, 58, 56, and 52 kD (lane 1). When these cells are cultured *in vitro,* they change their pattern of keratins and bands of 56, 50, and 46 kD are seen (lane 2). This pattern is indistinguishable from that produced by the cells upon malignant transformation (lane 3). However, in a liver metastasis of the esophageal carcinoma, the pattern of keratins was found to change again, with new keratins of 56, 46, and 40 kD being synthesized (lane 4).

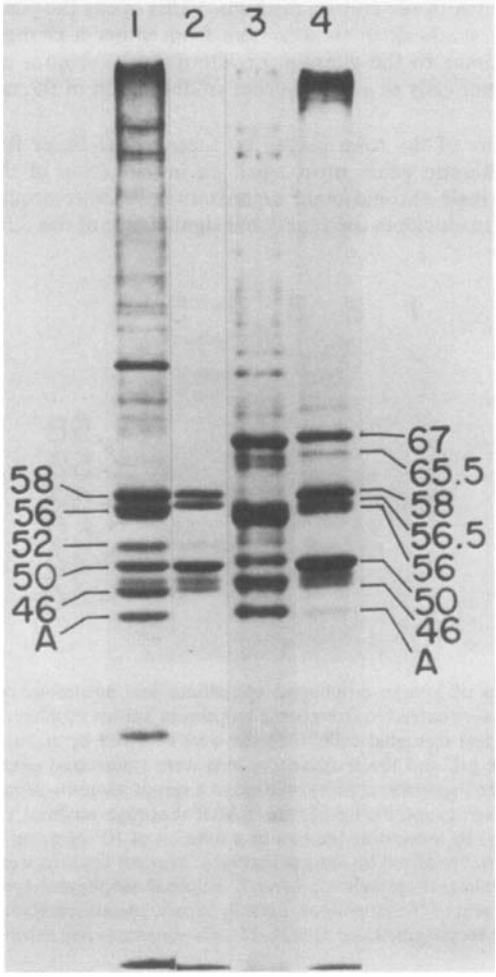


FIGURE 4. Synthesis of human keratins from mRNA of differentiating epidermis and of basal epidermal cells. Poly(A)$^+$ RNAs from whole thigh skin and from cultured basal epidermal cells were purified and translated *in vitro* in the presence of [^{35}S]methionine. The synthesized products were immunoprecipitated and resolved by SDS-polyacrylamide gel electrophoresis. Lanes 1 and 3:[^{35}S]methionine-labeled extracts from basal cells and epidermis, respectively. Lanes 2 and 4: Antikeratin immunoprecipitates of translated products of the mRNAs extracted from basal cells and epidermis, respectively.

We have shown that at least for cells that have been cultured from squamous cell carcinomas of the tongue and epidermis, the altered pattern of keratins produced on malignant transformation of these cells is transient and is influenced profoundly by the levels of vitamin A in the culture medium.[71] This opens the possibility that altered patterns of keratin synthesis in tumors stem from either a change in the sensitivity of the malignant tissue to the vitamin, or alternatively, from a change in the environment of the tumor cells as a consequence of disruption of the normal organization of the tissue.

The exact nature of the roles played by these extracellular factors in regulating the expression of keratin genes must await an investigation of the structure of the keratin genes and their chromosomal organization. Further sequencing will also be necessary to begin to elucidate the functional significance of the differential expression

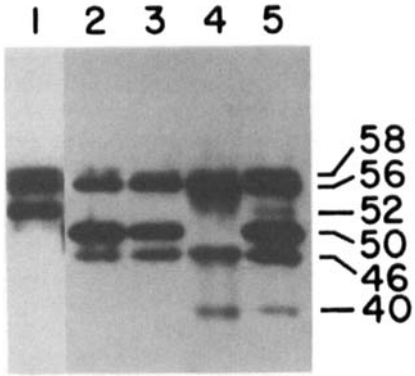


FIGURE 5. Keratins of human esophageal epithelium and squamous cell carcinoma of the esophagus. Keratins were extracted from tissue biopsies of human esophagus, esophageal cancer, and cultured esophageal epithelial cells. Proteins were resolved by electrophoresis through an 8.5% polyacrylamide gel, and the resolved proteins were transferred electrophoretically to nitrocellulose paper. The paper was then hybridized in a serum albumin-saline solution containing 1:100 dilution of antiserum specific for keratin.[12] After thorough washing, the keratins were then indirectly radiolabeled by incubating the blot in a solution of 10^5 cpm/ml ^{125}I-labeled S. aureus protein A. Bands were visualized by autoradiography. Human keratins were from the following sources: Lane 1, esophageal epithelium; Lane 2, cultured esophageal epithelial cells; Lane 3, squamous cell carcinoma of the esophagus; Lane 4, hepatic metastasis from a primary squamous cell carcinoma of the esophagus; Lane 5, SCC-15 cells squamous cell carcinoma of the tongue.[26]

of these genes. Nonetheless, it is clear that many of the mysteries of the most variable cytoskeletal component in higher eukaryotic organisms have begun to unravel. The field of keratin filaments appears to be at the threshold of elucidating the functional significance of the multiplicity of subunits that can all assemble into the abundant 8 nm filaments that form the structural web within an epithelial cell.

ACKNOWLEDGMENT

We thank Ms. Claire Doyle for her expeditious typing of this manuscript.

REFERENCES

1. TEZUKA, T. & I. M. FREEDBERG. 1972. Epidermal structural proteins. II. Isolation and purification of tonofilaments of the newborn rat. Biochim. Biophys. Acta **263:** 382-396.
2. BADEN, H. P., L. A. GOLDSMITH & B. FLEMING. 1973. The polypeptide composition of epidermal prekeratin. Biochim. Biophys. Acta **317:** 303-311.
3. STEINERT, P. M. & W. W. IDLER. 1975. The polypeptide composition of bovine epidermal α-keratin. Biochem. J. **151:** 603-614.
4. HUANG, L.-Y., I. B. STERN, J. A. CLAGETT & E. Y. CHI. 1975. Two polypeptide chain constituents of the major protein of the cornified layer of newborn rat epidermis. Biochemistry **14:** 3573-3580.
5. INOUE, N., K. FUKUYAMA & W. L. EPSTEIN. 1976. Immunochemical studies of proteins in epidermal cornified cells of human and newborn rat. Biochim. Biophys. Acta **439:** 95-106.
6. DALE, B. A., I. B. STERN, M. RABIN & L.-Y. HUANG. 1976. The identification of fibrous proteins in fetal rat epidermis by electrophoretic and immunologic techniques. J. Invest. Dermatol. **66:** 230-235.
7. CULBERTSON, V. B. & I. M. FREEDBERG. 1977. Isolation and characterization of the α-helical proteins from newborn rat. Biochim. Biophys. Acta **490:** 178-191.
8. BRYSK, M. M., R. H. GRAY & I. A. BERNSTEIN. 1977. Tonofilament protein from newborn rat epidermis. J. Biol. Chem. **252:** 2127-2133.
9. SUN, T.-T. & H. GREEN. 1978. Keratin filament of cultured human epidermal cells. J. Biol. Chem. **253:** 2053-2060.
10. TSENG, S. C. G., M. J. JARVINEN, W. G. NELSON, J. N. HUANG, J. WOODCOCK-MITCHELL & T.-T. SUN. 1982. Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. Cell **30:** 361-372.
11. MOLL, R., W. W. FRANKE, D. S. SCHILLER, B. GEIGER & R. KREPLER. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors, and cultured cells. Cell **31:** 1-24.
12. FUCHS, E. & 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.
13. CREWTHER, W. G., A. S. INGLIS & N. M. MCKERN. 1978. Amino acid sequences of α-helical segments from S-carboxymethylkerateine-A. Biochem. J. **173:** 365-371.
14. GOUGH, K. H., A. S. INGLIS & W. G. CREWTHER. 1978. Amino acid sequences of α-helical segments from S-carboxymethylkerateine-A. Biochem. J. **173:** 373-385.
15. FUCHS, E., S. COPPOCK, H. GREEN & D. CLEVELAND. 1981. Two distinct classes of epidermal keratin genes and their evolutionary significance. Cell **27:** 75-84.
16. HANUKOGLU, I. & E. FUCHS. 1983. The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. Cell **33:** 915-924.
17. FUCHS, E. & H. GREEN. 1980. Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell **19:** 1033-1042.
18. MILSTONE, L. M. & J. MCGUIRE. 1981. Different polypeptides form the intermediate filaments in bovine hoof and esophageal epithelium and in aortic endothelium. J. Cell Biol. **88:** 312-316.
19. FRANKE, W. W., D. L. SCHILLER, R. MOLL, S. WINTER, E. SCHMID, I. ENGELBRECHT., H. DENK., R. KREPLER & B. PLATZER. 1981. Diversity of cytokeratins: differentiation-specific expression of cytokeratin polypeptides in epithelial cells and tissues. J. Mol. Biol. **153:** 933-959.
20. KIM, K. H., J. G. RHEINWALD & E. V. FUCHS. 1983. Tissue-specificity of epithelial keratins: differential expression of mRNAs from two multigene families. Mol. Cell. Biol. **3:** 494-502.
21. SKERROW, D. & I. HUNTER. 1978. Protein modifications during the keratinization of normal and psoriatic human epidermis. Biochim. Biophys. Acta **537:** 474-484.
22. JACKSON, B. W., C. GRUND, S. WINTER, W. W. FRANKE & K. ILLMENSEE. 1981. Differentiation **20:** 203-216.

23. BANKS-SCHLEGEL, S. P. R. SCHLEGEL & G. S. PINKUS. 1981. Keratin protein domains within the human epidermis. Exp. Cell Res. **136:** 465-469.
24. BANKS-SCHLEGEL, S. P. 1982. Keratin alterations during embryonic epidermal differentiation: a presage of adult epidermal maturation. J. Cell. Biol. **93:** 551-559.
25. MOLL, R., I. MOLL & W. WIEST. 1983. Changes in the pattern of cytokeratin polypeptides in epidermis and hair follicles during skin development in human fetuses. Differentiation **23:** 170-178.
26. WU, Y. J. & J. G. RHEINWALD. 1981. A new small (40 dk) keratin filament protein made by some cultured human squamous cell carcinomas. Cell **25:** 627.
27. MOLL, R., R. KREPLER & W. W. FRANKE. 1983. Complex cytokeratin polypeptide patterns observed in certain human carcinomas. Differentiation **23:** 256-269.
28. FUCHS, E., M. P. GRACE, K. H. KIM & D. MARCHUK. 1984. Differential expression of two classes of keratins in normal and malignant epithelial cells and their evolutionary conservation. Cold Spring Harbor Symp. Proliferation Cancer. March 1984.
29. NELSON, W. G. & T.-T. SUN. 1983. The 50- and 58-kdalton keratin classes as molecular markers for stratified squamous epithelia: cell culture studies. J. Cell. Biol. **97:** 244-251.
30. LEE, L. D. & H. P. BADEN. 1976. Organization of the polypeptide chains in mammalian keratin. Nature **264:** 377-379.
31. STEINERT, P. M., W. W. IDLER & S. B. ZIMMERMAN. 1976. Self-assembly of bovine epidermal keratin filaments in vitro. J. Mol. Biol. **108:** 547-567.
32. MILSTONE, L. M. 1981. Isolation and characterization of two polypeptides that form intermediate filaments in bovine esophageal epithelium. J. Cell. Biol. **88:** 317-322.
33. CRICK, F. H. C. 1953. The packing of α-helices: simple coiled-coils. Acta Cryst. **6:** 689-697.
34. PAULING, L. & R. B. COREY. 1953. Compound helical configurations of polypeptide chains: structure of proteins in the α-keratin type. Nature **171:** 59-61.
35. SKERROW, D., G. MATOLTSY & M. MATOLTSY. 1973. Isolation and characterization of the helical regions of epidermal prekeratin. J. Biol. Chem. **248:** 4820-4826.
36. STEINERT, P. M. 1978. Structure of the three-chain unit of bovine epidermal keratin filament. J. Mol. Biol. **123:** 49-70.
37. MCLACHLAN, A. D. 1978. Coiled-coil formation and sequence regularities in the helical regions of α-keratin. J. Mol. Biol. **124:** 297-304.
38. AHMADI, B. & P. T. SPEAKMAN. 1978. Superimidate crosslinking shows that a rod-shaped, low cysteine, high helix protein prepared by limited proteolysis of reduced wool has four protein chains. FEBS Lett. **94:** 365-367.
39. QUINLAN, R. A. & W. W. FRANKE. 1982. Heteropolymer filaments of vimentin and desmin in vascular smooth muscle tissue and cultured baby hamster kidney cells demonstrated by chemical crosslinking. Proc. Natl. Acad. Sci. USA **79:** 3452-3456.
40. GEISLER, N. & K. WEBER. 1982. The amino acid sequence of chicken muscle desmin provides a common structural model for intermediate proteins including the wool α-keratins. EMBO J. **1:** 1649-1656.
41. AEBI, I., W. E. FOWLER, P. REW. & T.-T. SUN. 1983. The fibrillar substructure of keratin filaments unraveled. J. Cell. Biol. **97:** 1131-1143.
42. HANUKOGLU, I. & E. FUCHS. 1982. The cDNA sequence of a human epidermal keratin: divergence of sequence but conservation of structure among intermediate filament proteins. Cell **31:** 243-252.
43. CREWTHER, W. G., L. M. DOWLING & A. S. INGLIS. 1980. Amino acid sequence data from a microfibrillar protein of α-keratin. Proc. 6th Quinquennial Intl. Wool Text. Conf. **2:** 79-81.
44. QUAX, W., W. V. EGBERTS, W. HENDRIKS, Y. QUAX-JEUKEN & H. BLOEMENDAL. 1983. The structure of the vimentin gene. Cell **35:** 215-233.
45. CHOU, P. Y. & G. D. FASMAN. 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47:** 45-148.
46. CHOU, P. Y. & G. D. FASMAN. 1979. Prediction of β-turns. Biophys. J. **26:** 367-383.
47. GARNIER, J., D. J. OSGUTHORPE & 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.
48. STEINERT, P. M., R. H. RICE, D. R. ROOP, B. L. TRUS & A. C. STEVEN. 1983. Complete

amino acid sequence of a mouse epidermal keratin subunit and implications for the structure of intermediate filaments. Nature **302:** 794-800.

49. McLACHLAN, A. D. & M. STEWART. 1975. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. J. Mol. Biol. **98:** 293-304.
50. PARRY, D. A. D., W. G. CREWTHER, R. D. FRASER & T. P. MacRAE. 1977. Structure of α-keratin: structural implications of the amino acid sequence of the type I and type II chain segments. J. Mol. Biol. **113:** 449-454.
51. McLACHLAN, A. D. & M. STEWART. 1982. Periodic charge distribution in the intermediate filament proteins desmin and vimentin. J. Mol. Biol. **162:** 693-698.
52. FRANKE, W. W., D. L. SCHILLER, M. HATZFELD & 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.
53. STEINERT, P. M., W. W. IDLER & R. D. GOLDMAN. 1980. Intermediate filaments of baby hamster kidney (BHK-21) cells and bovine epidermal keratinocytes have similar ultrastructures and subunit structures. Proc. Natl. Acad. Sci. USA **77:** 4534-4538.
54. GEISLER, N. & K. WEBER. 1981. Comparison of the protein of two immunologically distinct intermediate-sized filaments by amino acid sequence analysis: desmin and vimentin. Proc. Natl. Acad. Sci. USA **78:** 4120-4123.
55. HONG, B.-S. & P. F. DAVIDSON. 1981. Isolation and characterization of a soluble, immunoactive peptide of glial fibrillary acidic protein. Biochim. Biophys. Acta **670:** 139-145.
56. GEISLER, N., U. PLESSMAN & K. WEBER. 1982. Related amino acid sequences in neurofilaments and non-neuronal intermediate filaments. Nature **296:** 448-450.
57. GEISLER, N., E. KAUFMANN, S. FISCHER, U. PLESSMAN & K. WEBER. 1983. Neurofilament architecture combines structural principles of intermediate filaments with carboxy-terminal extensions increasing in size between triplet proteins. EMBO J. **2:** 1295-1302.
58. GEISLER, N. & K. WEBER. 1983. Amino acid sequence data on glial fibrillary acidic protein (GFA); implications for the subdivision of intermediate filaments into epithelial and non-epithelilal members. EMBO J. **2:** 2059-2063.
59. STEINERT, P. M., W. W. IDLER, F. CABRAL, M. GOTTESMAN & R. GOLDMAN. 1981. *In vitro* assembly of homopolymer and copolymer filaments from intermediate filament subunits of muscle and fibroblastic cells. Proc. Natl. Acad. Sci. USA **78:** 3692-3696.
60. GEISLER, N. & K. WEBER. 1981. Self-assembly *in vitro* of the 68,000 molecular weight component of the mammalian neurofilament triplet proteins into intermediate-sized filaments. J. Mol. Biol. **151:** 565-571.
61. LEWIS, S. A., J. M. BALCAREK, V. KREK, M. SHELANSKI & N. COWAN. Sequence of a cDNA clone encoding mouse glial fibrillary acidic proteins: structure and conservation of intermediate filaments. Proc. Natl. Acad. Sci. USA **81:** 2743-2746.
62. DOWLING, L. M., D. A. D. PARRY & L. G. SPARROW. 1983. Structural homology between α-keratin and the intermediate filament proteins desmin and vimentin. Biosci. Rep. **3:** 73-78.
63. MARCHUK, D., S. McCROHON & E. FUCHS. 1984. The complete amino acid sequence of a human type I keratin as determined by DNA sequence analysis: isolation and characterization of the gene encoding the 50 kd human epidermal keratin.
64. FUCHS, E. & I. HANUKOGLU. 1983. Unravelling the structure of the intermediate filaments. Cell **34:** 332-334.
65. GEISLER, N., E. KAUFMANN & K. WEBER. 1982. Protein chemical characterization of three structurally distinct domains along the protofilament unit of desmin 10 nm filaments. Cell **30:** 277-286.
66. FUCHS, E. & H. GREEN. 1979. Multiple keratins of cultured human cells are translated from different mRNA molecules. Cell **17:** 573-582.
67. SCHWEIZER, J. & K. GOERTTLER. 1980. Synthesis *in vitro* of keratin polypeptides directed by mRNA isolated from newborn and adult mouse epidermis. Eur. J. Biochem. **112:** 243-249.
68. ROOP, D. R., P. HAWLEY-NELSON, C. K. CHENG & S. H. YUSPA. 1983. Keratin gene expression in mouse epidermis and cultured epidermal cells. Proc. Natl. Acad. Sci. USA **80:** 716-720.

69. SCHWEIZER, J. & H. J. WINTER. 1983. Keratin biosynthesis in normal mouse epithelia and in squamous cell carcinomas. J. Biol. Chem. **258:** 13268-13272.
70. MAGIN, T. M., J. L. JORCANO & W. W. FRANKE. 1983. Translational products of mRNAs coding for non-epidermal cytokeratin. EMBO J. **2:** 1387-1392.
71. KIM, K. H., D. MARCHUK & E. FUCHS. 1984. A commitment to terminal differentiation in keratinocytes is associated with a shift to the synthesis of unusually large keratins. J. Cell Biol. **99:** 1872-1877.
72. FUCHS, E. & H. GREEN. 1981. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell **25:** 617-625.
73. VAN SCOTT, E. J. & R. P. REINERTSON. 1961. The modulating influence of stromal environment on epithelial cells studied in human autotransplants. J. Invest. Dermatol. **36:** 109-131.
74. SENGEL, P. 1964. The determination of the differentiation of the skin and the cutaneous appendages of the chick embryo. *In* The Epidermis. W. Montagna & W. C. Lobitz, Eds.: 14-34. Academic Press. New York.
75. BILLINGHAM, R. E. & W. K. SILVERS. 1968. Dermoepidermal interactions and epithelial specificity. *In* Epithelial-Mesenchymal Interactions. R. Fleischmajer & R. E. Billingham, Eds.: 252-266. Williams and Wilkins. Baltimore.
76. GREEN, H. 1981. Cyclic AMP in relation to proliferation of the epidermal cell: a new review. Cell **15:** 801-811.
77. RHEINWALD, J. G. & H. GREEN. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell **6:** 331-343.
78. RHEINWALD, J. G. & H. GREEN. 1977. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature **265:** 421-424.
79. BANKS-SCHLEGEL, S. P. & C. C. HARRIS. 1984. Aberrant expression of keratin proteins and crosslinked envelopes in human esophageal carcinomas. Cancer Res. **44:** 1153-1157.
80. GRACE, M. P., K. H. KIM, L. TRUE & E. V. FUCHS. 1985. Keratin expression in esophageal epithelium and squamous cell carcinoma of the esophagus. Cancer Res. **45:** 841-846.