Chapter 32 Epidermal α-Keratins: Structural Diversity and Changes During Tissue Differentiation

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32.1 Introduction

The epidermis and its appendages, including hair, wool, nail, horn, hoof and porcupine quill represent the major protective covering of vertebrate organisms. To fulfull this function, these tissues include many different types of biochemical adaptations in their biological designs. Yet their predominant features are bundles of 8-nm keratin filaments. The longitudinal network formed by filaments is probably more responsible for their strength and special characteristics than are any other of cytoplasmic components. Despite the diverse morphologies, it is now clear that the keratin filaments are comprised of subunits that constitute a family of related proteins known as α-keratins. In epidermal cells, these proteins, henceforth named cytoskeletal keratins, assemble into 8-nm filaments and form a seemingly irregular interwoven mesh throughout the cytoplasm. In epidermal appendages such as hair and wool, these proteins, henceforth named microfibrillar keratins, form very orderly arrays of microfibrils embedded in an amorphous matrix. The proteins that constitute the matrix in epidermal appendages are often called keratins; however, since these "matrix keratins"

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are different from the microfibrillar α-keratins both in terms of their sizes (6–20 K vs. 40–70 K for α-keratins) and their primary and secondary structures, we shall not consider them here. In this chapter, we shall review recent studies on the structural characteristics of the keratin subunits that constitute the backbone of the 8-nm filaments, and the changes in the spectrum of these subunits during terminal differentiation and embryonic development of the epidermis.

32.2   Sequence and Structural Relationship of α-Keratin Filament Subunits

32.2.1   Classification of Keratins into Two Distinct Groups and Their Evolutionary Conservation

In the past 15 years, the most extensively characterized keratins have been the microfibrillar keratins of sheep wool and the epidermal keratins of human, rodent and cow. The studies on the keratins of these diverse sources have indicated that, unlike the two other types of cytoskeletal proteins, actin and tubulin, the keratins vary widely in their size ($M_r = 40$–70 K). Nonetheless, they display similarities in their structure as evidenced by analysis of amino acid compositions, one-dimensional peptide mapping, and immunological cross-reactivity (Jones 1975, 1976; Steinert and Idler 1975; Fuchs and Green 1978; Dale et al. 1976; Weber et al. 1980; Bowden and Cunliffe 1981). Partial amino acid sequence data on wool microfibrillar keratins indicated that despite certain common properties among all keratins, at least two of the keratins share only a low ($< 30\%$) homology with each other (Crowther et al. 1978, 1980a, 1980b; Gough et al. 1978). Subsequently, as mRNAs coding for the epidermal keratins were isolated and cloned as cDNAs (Fuchs et al. 1981; Roop et al. 1983), it was discovered that, in fact, most if not all of the five to seven human epidermal cytoskeletal keratins could be subdivided into two distinct groups of sequences according to the ability of their corresponding mRNAs to cross-hybridize with one another (Fuchs et al. 1981; Kim et al. 1983, 1984). Upon the discovery that the two respective wool keratins share striking sequence similarities with the two classes of human epidermal keratin sequences, the terminology used for the two types of microfibrillar keratin fragments was adopted for cytoskeletal keratins (Hanukoglu and Fuchs 1983). Hence, these two groups of keratin sequences have been classified as type I and type II keratins.

The type I and type II keratins are present in all vertebrate species and each type is encoded by a small multigene family. The number of genes coding for each type of keratin varies between two and ten across species. For example, in humans there appear to be about ten genes for the type I and ten genes for the type II keratins, whereas for the primitive vertebrate hagfish, there appear to be only one or two genes for each type (Fuchs et al. 1981; Fuchs and Marchuk 1983). As judged by DNA-DNA hybridizations, DNA sequences that are highly homologous to cloned human epidermal type I and type II keratin cDNAs are present in all vertebrates (Fuchs et al. 1981; Fuchs and Marchuk 1983).
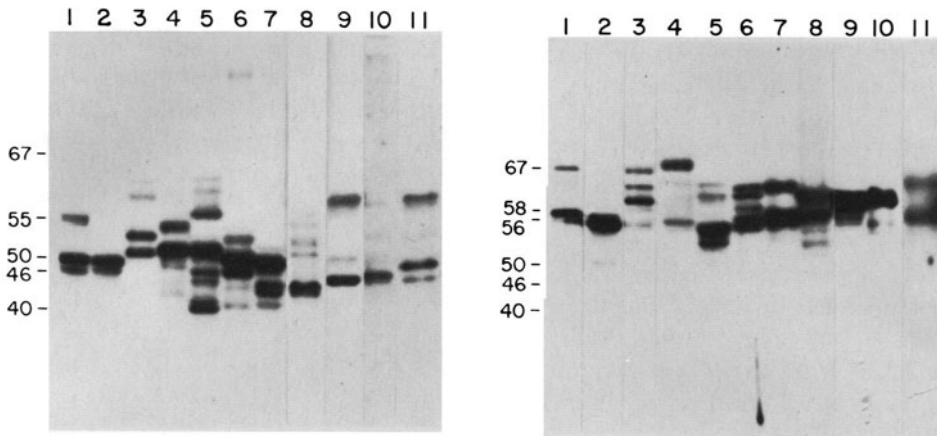
Fig. 1. Type I (*left*) and type II (*right*) keratins are present in the epidermis of all vertebrates. Antibodies were raised against electrophoretically purified M_r 50 K (type I) and M_r 56 K (type II) human keratins. These antisera were used in immunoblot analysis to detect the presence of immunoreactive forms of both classes of keratins in other vertebrate epidermal keratinocytes. (*Left*) Immunoblot with anti-type I keratin antisera. (*Right*) Immunoblot with anti-type II keratin antisera. Tracks with different samples of vertebrate keratins: *1* whole human epidermis; *2* cultured human epidermal cells; *3* rat; *4* mouse; *5* hairless mutant mouse; *6* rabbit; *7* bovine snout; *8* chicken; *9* turtle; *10* frog; *11* catfish fin. (M_r s are shown $\times 10^{-3}$). (Fuchs and Marchuk 1983)

The universal presence of type I and type II epidermal keratins in all vertebrate skins has been confirmed by their ability to cross-react with antibodies specific for human epidermal type I and type II keratins (Fig. 1). In a duplicate immunoblot analysis with the two respective antikeratin antisera, it was found that every vertebrate epidermis expressed at least one member of each of the two types of keratins (Fuchs and Marchuk 1983). All vertebrate keratins showed crossreactivity with one or the other of these two antisera. However, in the absence of detailed sequence information on multiple keratins from divese sources, the possibility cannot yet be excluded that there might be additional sequence classes representing minor forms of keratins.

32.2.2 Sequences and Characteristics of Keratin Subunits

At present, the sequences of two wool microfibrillar keratins, two human epidermal cytoskeletal keratins and one mouse epidermal cytoskeletal keratin are known (Fig. 2) (Crewther et al. 1978, 1980a, 1980b; Gough et al. 1978; Dowling et al. 1983; Hanukoglu and Fuchs 1982, 1983; Steinert et al. 1983). A comparison of these sequences coupled with the data obtained from biochemical experiments using cloned keratin cDNAs and antibodies specific to each type of keratin have provided valuable information on the structural features of the two types of keratin subunits. The following major similarities and differences have been observed among the type I and type II keratins:

(1) The M_r ranges of the keratins in the two classes are largely non-overlapping. The type I keratins are generally small (M_r 40–55 K), whereas the type II keratins are larger (M_r 56–70 K). The mouse type I keratin (M_r 59 K) shown in Fig. 2 appears to be an exception among the vertebrate type I keratins because of its rather large size (see Fuchs et al. 1981; Fuchs and Marchuk 1983).

(2) The type I keratins generally show isoelectric points that are more acidic than those of type II keratins (P_i 4.5–5.5 vs. 6.5–7.5) (for a review see Moll et al. 1982a). This observation remains unexplained, since the sequence and amino acid composition differences between these two types of keratins do not readily explain this isoelectric point difference (Table 1).

(3) The central approximately 300 residue long portion of all keratin sequences can be aligned for optimal homology without any or only a few (1–3) gaps (Fig. 2). When a comparison was made between any type I and type II keratin, it was found that they share only a low (<30%) sequence homology in this central region, even within the same species (Fig. 3 A). However, within a particular type of keratin, both epidermal cytoskeletal and wool microfibrillar keratins share a relatively high homology (>60%) in this central region, even across species (Fig. 3 B).

(4) Even though there is only a low degree of homology between the two types of keratin sequences, the secondary structures of the central regions of all keratins seem to be remarkably conserved. The secondary structure prediction analyses indicate that within this central region, there are four richly α-helical domains. These are marked by bars over the sequences in Figs. 2 and 3. For the three unmarked regions demarcating the α-helical domains, β-turns are predicted with a high degree of probability in all sequences. The first two of these β-turn regions contain proline(s) in some, but not all sequences.

(5) Despite evolutionary divergence of sequence, the amino acid compositions of the total central region, as well as those of individual helical domains of both types of keratin, have remained highly similar. Some degree of conservation appears to arise from the constraints placed on maintaining α-helicity in this region. For example, 25–30% of the residues in this domain are Glu and Leu, both of which favour α-helical structures (Tables 1, 2). Superimposed on these structural constraints are additional conservations, which most likely reflect sequences that are essential for the assembly of the filament.

(6) The four helical domains (marked as I, II, III, and IV in Figs. 2 and 3 and Table 2) are predicted to be nearly constant in size in all keratins and they are approximately 30–40, 100, 35–40, and 100 residues long respectively.

Although amino acid sequence homology is higher within all predicted helical domains than it is in the non-helical regions, it is especially prominent in domain III and in the 3′ end of domain IV.

(7) Within the helical domains, charged and hydrophobic residues are conserved more frequently and many substitutions for these residues represent conservative replacements, e.g. Asp(−) for Glu(−), or Arg(+) for Lys(+).

(8) The size differences among keratins in general, and between type I and type II keratins in particular, seem to arise from differences in the non-helical amino and carboxy terminal portions of the proteins rather than in the structurally conserved central α-helical region (Figs. 2 and 3). These terminal regions

Amino terminus

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WT-I  :    1  SFNFCLPNLSFRSSCSSRPCVPSSCCGTTLPGACNIPANVGSCNWRCEGSFDGN
MT-I  :    1  SVLYCSSSKQFSSSRSGGGGGGGSVRVSSTRGSLGGLSSGGFSGGSFSRGSSGGGGCFGGSSGGYGGFGGGGSFGGGYGGSSFGGGY  87
HT-I  :   88  GGSSFGGGYGGGSFGGGSFGGGSFGGGFGGGGFGGGGFGDGGGLLLSGN
HT-II :    1  ...GLGGGYGGGFSSSSSSFGSGFGGGYGGGLGTGLGGGFGGGFAGGDGLLVGS
HT-II :          ...
WT-II :          ...
```

Helical domain I

```
WT-I  :   55  EKETMQFLNDRLASYLEKVRQLERENAELESRILERSQQQEPLVCPN
MT-I  :  142  GRVTMRNLNDRLASYMDKVRALEESNYELEGKIKEVVREARQLKPREPRD
HT-I  :   52  EKVTMQNLNDRLASYLDKVRALEEANADLEVKIRDWYQRQRPAEIKD
HT-II :    1  ...............................QNL
WT-II :    1  .............................RQCCESNL
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Helical domain II

```
WT-I  :  102  YQSYFRTIEELQQKILCAKSENSRLVIEIDNAKLASDDFRTKYESERSLRQLVESDINSLRRILDELTLCKSNLEAEVESLKEELLCLKQNHEEVNTLRS
MT-I  :  192  YSKYYKTIECLKGQILTLTTDNANVLLQIDNARLAADDFFRLKYENEVTLRQSVEADINGLRRVLDELTLSQSVLELQIESLNEELAYLKKNLEEEMRDLQN
HT-I  :   99  YSPYFKTIEDLRNKILTATVDNANVLLQIDNARLAADDFRTKYETELNLRMSVEADINGLRRVLDELTLARADLEMQIESLKEELAYLKKNHEEMNALRG
HT-II :    4  EPLFEQYINNLRRQLDSIVGERGRLDSELRGMQDLVEDFKNKYEDEINKRTAAENEFVTLKKDVDAAYMNKVELQAKADTLTDEINFLRALYDAELSQMQT
WT-II :    9  EPLFEGYIETLRREAECVEADSGRLSSELNHVQEVLEGYKKKYEEEVALRATAENEFVALKKDVDCAYVRKSDLEANVEALIQEIDFLRRLYEEIRVLQA
```

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WT-I  :  203  QLGDRLNVE   VDAAPTV
MT-I  :  293  VSTGDVNVE   MNAAPGV
HT-I  :  200  QVGGDVNVE   MDAAPGV
HT-II :  105  HISDTSVVLS  MDNNRNL
WT-II :  110  NISDTSVIVSKMDNSRDL
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Helical domain III

```
WT-I  : 219 DLNRVLN ETRAQYEALVETNRRDVEEWYIRQTEELNKQVVSSSEQLQCNQE
MT-I  : 309 DLTQLLN NMRNQYEQLAEKNRKDAEEWFNQKSKELTTEIDSNIAQMSSHKS
HT-I  : 216 DLSRILN EMRDQYEKMAEKNRKDAEEWFFTKTEELNRFVATNSELVQSGKS
HT-II : 122 DLDSIIA EVKAQYEEIAQRSRAEAESWYQTKYEELQVTAGRHGDDLRNTKQ
WT-II : 128 NMDCIVAEEIRAQYDDIASRSRAEAESWYRSKCEEIKATVIRHGETLRRTKE
```

Helical domain IV

```
WT-I  : 270 EIIELRRTVNALQVELQAQHNLRDSLENTLTETEARYSCQLNQVQSLISNVESQLAEIRGDLERQNQEYQVLLDVRARLECEINTYRGLLDSEDCKL
MT-I  : 360 EITELRRTVQGLEIELQSQLALKQSLEASLAETVESLLRQLSQIQSQISALEEQLQQIRAETECQNAEYQQLLDIKTRLENEIQTYRSLLEGEGSSS
HT-I  : 267 EISELRRTMQNLEIELQSQLSMKASLENSLEETKGRYCMQLAQIQEMIGSVEEQLAQLRCEMEQQNQEYKILLDVKTRLEQEIATYRRLLEGEDAHL
HT-II : 173 EIAEINRMIQRLRSESDHVKKQCANLQAAIADAEQRGEMALKDAKNKLEGLEDALQKAKQDLARLLKEYQELMNVKLALDVEIATYRKLLEGEECRL
WT-II : 180 EINELNRVIQRRTAEVE.......... . . . . . . . .KAKQNMACLLKEYQEVMNSKLGLDIEIATYRRLLEGEEQRL
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Carboxy terminus

```
WT-I  : 367 ...ACGKPLTPCISSPCAPAAPCTTCVVPSSCGRRY
MT-I  : 457 GGGGGRRGGSGGGSYGGSSGGGSYGGSSGGGSYGGSSGGGSYGGSSGCGRGGGSGSGGGYGGGGSSSGGAGRGGGSGSGGGYGGGSSGRRGGSGGGF 553
        554 SGTSGGGDQSSKGPRY
HT-I  : 364 SSSQFSSGSGQSSRDVTSSSRQIRTKVMDVHDGKVVSTHEQVLRTKN
HT-II : 270 NGEGVGQVNISVVQSTVSSGYGGASGVGSGLGLGGGSSYSYGSGLGVGGGFSSSSGRAIGGGLSSVGGGSSTIKYTTSSSSRKSYKH
WT-II :     CQGV..
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Fig. 2. The amino acid sequences of type I and type II cytoskeletal and microfibrillar keratins and the common location of their predicted helical domains. The sequences are from the following sources: (1) *WT-I* type I microfibrillar keratin from sheep wool (Crewther et al. 1978, 1980b; Gough et al. 1978; Dowling et al. 1983); (2) *MT-I* type I cytoskeletal keratin from mouse epidermis ($M_r=59$ K) (Steinert et al. 1983); (3) *HT-I* type I cytoskeletal keratin from human epidermal cells ($M_r=50$ K) (Hanukoglu and Fuchs 1982); (4) *HT-II* type II cytoskeletal keratin from human epidermal cells ($M_r=56$ K) (Hanukoglu and Fuchs 1983); (5) *WT-II* type II microfibrillar keratin from sheep wool (Gough et al. 1978; Dowling et al. 1983). *Dots* indicate missing sequence information. In cases where the sequence of the amino terminus is not known, the *numbering on the left side* of the sequences starts with the first known amino terminal residue of the longest segment. With the exception of WT-II, the sequences extend to the last carboxy terminal residue of the respective protein. The *gaps in the sequences* indicate gaps introduced in order to align the sequences for optimal homology. The position of the helical domains is based on computerized secondary structure prediction analyses using the Chou and Fasman (1978, 1979) and Garnier, Osguthorpe and Robson (1978) methods as previously described (Hanukoglu and Fuchs 1982, 1983).

Table 1. The amino acid compositions of the different structural domains of epidermal (cytoskeletal) and wool (microfibrillar) keratins

	Amino terminus			Central region					Carboxy terminus			
	WT-I	MT-I	HT-I[1]	WT-I	MT-I	HT-I	HT-II[1]	WT-II[1]	WT-I[1]	MT-I	HT-I	HT-II
Ala	3.7	0.0	2.0	4.8	6.3	8.0	10.0	8.4	12.1	0.9	0.0	2.3
Arg(+)	5.6	2.8	0.0	9.0	7.0	7.7	7.1	9.7	6.1	6.2	8.7	2.3
Asn	11.1	0.7	0.0	7.1	6.7	6.1	5.9	4.6	0.0	0.0	2.2	2.3
Asp(−)	1.9	0.7	2.0	4.8	4.8	6.4	8.2	5.1	0.0	0.9	6.5	0.0
Cys	14.8	2.1	0.0	2.6	0.6	0.6	0.7	3.0	18.2	0.9	0.0	0.0
Gln	0.0	0.7	0.0	8.7	8.6	6.4	7.1	3.8	0.0	0.9	8.7	2.3
Glu(−)	1.9	0.0	0.0	15.4	13.3	14.1	11.9	16.5	0.0	0.0	2.2	1.1
Gly	9.3	49.6	52.9	1.0	2.9	2.9	3.0	2.5	6.1	58.4	4.3	29.5
His	0.0	0.0	0.0	0.6	0.3	0.6	1.1	0.8	0.0	0.0	4.3	1.1
Ile	1.9	0.0	0.0	4.2	4.8	4.5	4.8	6.3	3.0	0.0	2.2	3.4
Leu	5.6	3.5	9.8	14.7	14.6	12.8	12.3	9.7	3.0	0.0	2.2	4.5
Lys(+)	0.0	0.7	0.0	3.5	5.4	6.1	7.4	5.9	3.0	0.9	6.5	3.4
Met	0.0	0.0	0.0	0.3	1.9	3.8	2.6	1.7	0.0	0.0	2.2	0.0
Phe	7.4	9.9	9.8	1.0	0.6	1.3	1.5	1.3	0.0	0.9	2.2	1.1
Pro	9.3	0.0	0.0	1.0	1.0	1.0	0.4	0.4	18.2	0.9	0.0	0.0
Ser	18.5	22.7	15.7	7.4	7.6	4.5	4.1	5.9	12.1	22.1	26.1	26.1
Thr	3.7	0.7	2.0	4.5	5.4	4.5	3.7	3.4	9.1	0.9	8.7	5.7
Trp	1.9	0.0	0.0	0.3	0.3	0.6	0.4	0.4	0.0	0.0	0.0	0.0
Tyr	0.0	3.5	3.9	2.9	3.2	3.2	3.3	3.8	3.0	6.2	0.0	5.7
Val	3.7	2.1	2.0	6.4	4.8	4.8	4.5	6.8	6.1	0.0	13.0	9.1

The values are presented as percentages and are based on the sequences shown in Fig. 1. The "Central region" represents the segment extending from the first amino acid of Helical Region I to the last amino acid of Helical Region IV

1 The composition is based on incomplete sequence for this region

Fig. 3. Matrix plot of homology between **A** a human 50 K type I(HT-I) and a 56 K type II ▷ epidermal cytoskeletal keratin (HT-II); and **B** the human type I(HT-I) and a wool microfibrillar type I keratin (WT-I). *Numbers on the axes* correspond to the residue numbers of the respective sequences in Fig. 2. The positions of the four predicted helical domains are similarly shown to correspond with Fig. 2 and are marked by bars *(I–IV)* along the residue axes. Note that the sequence of the amino terminal end and the first predicted helical region *(I)* of HT-II are not yet known, although its structure is expected to be similar to that of HT-I (Hanukoglu and Fuchs 1983). 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 = 35%. 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 = 90–100%. Note that the regions of greatest homology in both A and B fall within the 4 helical domains. In addition, the carboxy termini (residues 280–325) of the human type I and type II epidermal keratins show high homology, both being rich in glycine and serine residues.
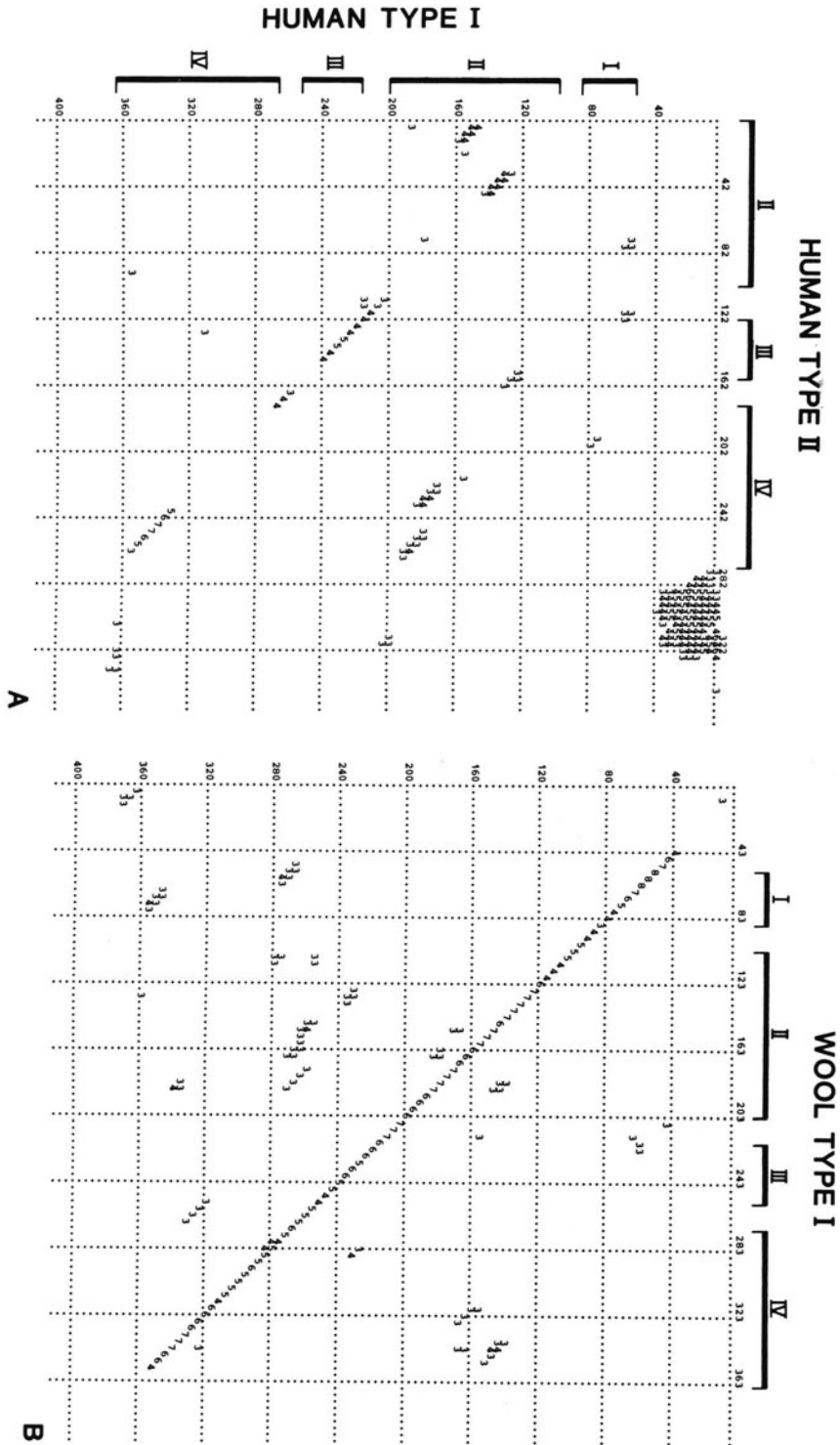
Table 2. The amino acid compositions of the helical domains in Type I and Type II mouse and human epidermal keratins

	MT-I				HT-I				HT-II		
	I	II	III	IV	I	II	III	IV	II	III	IV
Ala	5.9	5.9	5.1	6.2	11.8	9.9	5.1	5.2	7.9	15.4	13.4
Arg(+)	11.8	5.9	5.1	6.2	8.8	7.9	10.3	7.2	6.9	5.1	7.2
Asn	8.8	7.9	12.8	2.1	8.8	7.9	7.7	3.1	6.9	0.0	4.1
Asp(−)	5.9	6.9	5.1	1.0	8.8	7.9	7.7	2.1	9.9	5.1	6.2
Cys	0.0	1.0	0.0	1.0	0.0	0.0	0.0	2.1	0.0	0.0	2.1
Gln	0.0	5.9	10.3	15.5	2.9	2.0	2.6	12.4	5.9	10.3	7.2
Glu(−)	11.8	11.9	15.4	16.5	11.8	11.9	20.5	17.5	11.9	17.9	13.4
Gly	5.9	2.0	0.0	3.1	0.0	2.0	0.0	3.1	3.0	0.0	3.1
His	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	1.0
Ile	2.9	5.0	2.6	7.2	2.9	5.0	2.6	6.2	4.0	7.7	5.2
Leu	11.8	18.8	12.8	17.5	14.7	15.8	7.7	15.5	11.9	5.1	15.5
Lys(+)	8.8	5.9	10.3	2.1	8.8	5.9	10.3	4.1	6.9	5.1	10.3
Met	5.9	1.0	2.6	0.0	2.9	3.0	5.1	5.2	3.0	0.0	3.1
Phe	0.0	1.0	2.6	0.0	0.0	2.0	5.1	0.0	4.0	0.0	0.0
Pro	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
Ser	5.9	5.0	2.6	11.3	2.9	3.0	2.6	6.2	3.0	7.7	2.1
Thr	2.9	5.9	7.7	6.2	2.9	5.9	5.1	4.1	5.0	5.1	1.0
Trp	0.0	0.0	2.6	0.0	0.0	0.0	2.6	0.0	0.0	2.6	0.0
Tyr	5.9	5.0	2.6	2.1	2.9	4.0	2.6	3.1	4.0	7.7	2.1
Val	5.9	5.0	0.0	2.1	8.8	4.0	2.6	2.1	5.0	5.1	3.1

The values are presented as percentages and are based on the sequences shown in Fig. 1

show a high degree of heterogeneity not only in their size, but also in amino acid composition and sequence, especially between cytoskeletal and microfibrillar keratins (Table 1). The carboxy termini of both types of epidermal keratins show some sequence homology and both are rich in glycine and serine residues (Fig. 3). However, the precise sequences within this region vary considerably and moreover, the carboxy terminus of the type II keratin is substantially larger than its type I counterpart.

32.2.3   A Comparison of the Cytoskeletal and Microfibrillar Type I and Type II Keratins

Many of the general characteristics noted in the previous section apply to both cytoskeletal and microfibrillar type I and type II keratins. However, the keratins that make up the microfibrils in such epidermal structures as hair, horn, and porcupine quill form highly ordered bundles of filaments, whereas in contrast, the epidermal keratins form comparatively loosely packed cytoskeletal bundles (for review see Fraser et al. 1972). Coupled with these obvious differences in gross morphologies, the ultrastructural differences suggest that the keratins that form microfibrils in epidermal appendages may differ from the keratins that form the cytoskeletal architecture in the epidermal cells. Indeed, the sequences

of human epidermal keratins and sheep wool keratins have revealed clear differences that may in large part account for tissue-specific variations in the properties of 8 nm keratin filaments.

The most obvious differences between cytoskeletal and microfibrillar keratins are localized at the amino and carboxy termini of these proteins (Fig. 3B). In these regions, both wool type I and type II keratins are rich in cysteine and proline (Table 1; Crewther et al. 1980a, 1980b; Dowling et al. 1983). In contrast, the termini of both human and mouse epidermal cytoskeletal keratins contain no or very few cysteines or prolines, but instead are rich in glycine and include a tandemly repeating pattern of three or four glycines frequently separated by clusters of serines (Fig. 2). While the central regions of both microfibrillar and cytoskeletal keratins of the same type are highly homologous, the only apparent sequence similarity at the amino and carboxy termini of these proteins is a relatively high ratio of serines in these segments (Fig. 3B and Table 1).

At present, the physical shape assumed by these terminal regions is unknown, but it is likely that future structural information will disclose novel and distinct features at the ends of these proteins. The richness of cysteines in the terminal regions of the microfibrillar keratins suggests that these residues are involved in S-S covalent bond formation between individual keratins. This aspect probably contributes greatly to the special characteristics and strength of the resulting keratin filaments.

32.2.4   Structural Significance of the Two Types of Keratins

Keratin filaments both in microfibrils and in cytoskeletal bundles are composed of protofibrils wherein each protofibril represents a polymer of polypeptide chains. At present, the number of keratin polypeptides that form the coiled-coil structure of a protofibril and the number of protofibrils that make up a filament have not been determined with certainty. It is also not known precisely how the individual polypeptide subunits interact to form the protofibril of the keratin filament. However, as has been first observed in wool microfibrillar keratins and later confirmed for other cytoskeletal keratin sequences, the helical domains of these proteins contain periodicities in the positioning of hydrophobic and charged residues (Fraser et al. 1976; Parry et al. 1977; Crewther et al. 1978; Elleman et al. 1978; Gough et al. 1978; McLachlan 1978; Hanukoglu and Fuchs 1982, 1983; Steinert et al. 1983; Dowling et al. 1983). It is thus currently thought that these residues define orderly surfaces alongside the helical domains wherein hydrophobic or complementary charge interactions between keratin polypeptides provide the driving force for their assembly into coiled-coil filaments.

Unlike actin filaments or microtubules, in vitro assembly of keratin filaments from purified protein subunits can be achieved with minimal requirements by a combination of purified subunits. Keratin filaments can be denatured in vitro with the aid of 8 M urea and a reducing agent. Upon subsequent removal of the denaturing agents, the filaments can spontaneously renature and reassemble into filaments in the absence of any auxiliary proteins or factors (Steinert

et al. 1976, 1979; Matoltsy et al. 1981). However, it is well documented that filament assembly cannot proceed unless at least two different keratin subunits are present (Lee and Baden 1976; Steinert et al. 1976; Milstone 1981).

Since the type I and type II keratins are coordinately expressed at all stages of differentiation and development, and since these sequences are coordinately conserved throughout evolution, it seems likely that these two types of subunits form the backbone of the protofibril of the 8 nm filament and that a member of each type may be required for filament assembly (Fuchs and Marchuk 1983; Kim et al. 1983). This concept has been supported by recent studies investigating the dissociation and subsequent renaturation of keratin filaments under different conditions of stringency (Franke et al. 1983). These studies showed that different keratin subunits interact with one another with varying strengths of association, and therefore, when present as a mixture, the subunits tend to segregate in pairs as the conditions for filament assembly are gradually lowered in stringency. In general, at least one member of the relatively small and acidic keratins (type I) was associated with at least one representative of the class of basic and large keratins (type II) and judged by the isoelectric focusing points of the resulting intermediate keratin subunit complexes.

Although these experiments argue strongly in favor of the pairing of type I and type II keratins within the cytoplasm of the epidermal cell, they do not necessarily indicate that the keratin filaments formed in vivo within a single cell are different in their subunit composition. It may be that in cells where more than one type I and type II keratin subunits are expressed, all subunits interact to form heteropolymeric 8 nm filaments. As monoclonal antibodies specific for each of the keratin subunits become available, this issue should be resolved unequivocally.

32.3   Changes in Keratin Subunits During Terminal Differentiation

Several laboratories have reported differences in size between the keratins of str. corneum and those of the living layers of the epidermis (Dale and Stern 1975; Dale et al. 1976; Baden and Lee 1978; Skerrow and Hunter 1978; Fuchs and Green 1980; Skerrow and Skerrow 1983). Moreover, it has been demonstrated that epidermal cells cultured under conditions that favour growth rather than differentiation produce a pattern of keratins distinct from that of the str. corneum (Sun and Green 1978). To examine the changes in the pattern of keratins during terminal differentiation, human skin was sectioned into 15 μm layers parallel to the surface of the skin and the keratin proteins were extracted from each layer (Fuchs and Green 1980). The keratins were resolved by SDS polyacrylamide gel electrophoresis and visualized by staining with Coomassie Blue (Fig. 4). Since the degree of differentiation attained by a cell can be approximated by its relative migration towards the skin surface, the pattern of keratins produced by a cell at each stage of differentiation can be obtained from sequential skin sections. In sections of pure dermis (track 1), a very weak band at 57 K was the only insoluble protein observed in the molecular weight range of the keratins. This protein is probably vimentin, an intermediate filament
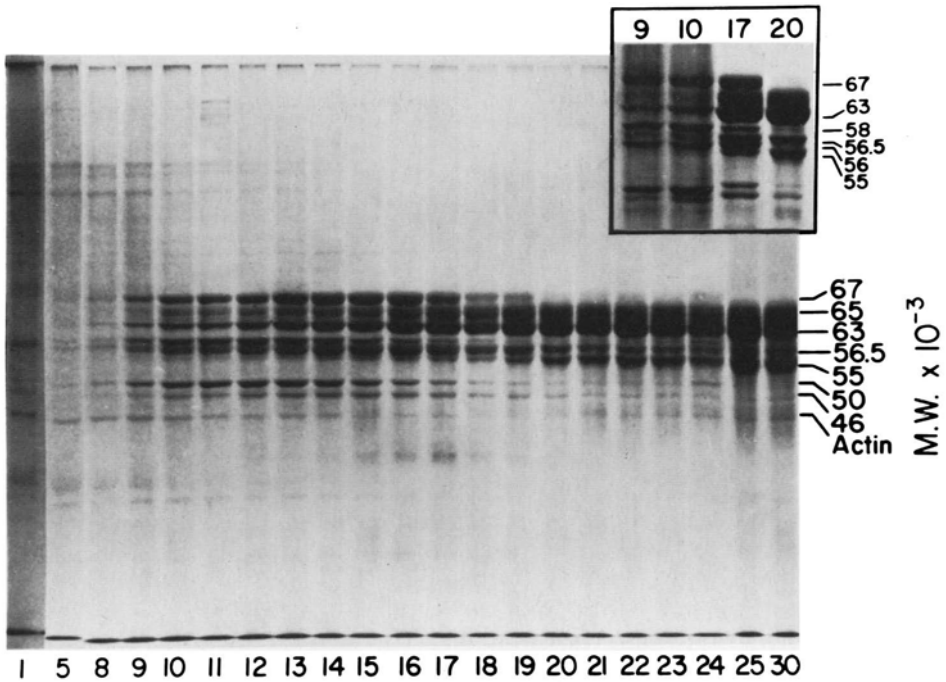
Fig. 4. Changes in the keratin subunits at different stages of terminal differentiation in human epidermis. A 3 mm square of human plantar skin was sliced into 15 µm sections parallel to the epidermal surface. The keratins were extracted from each section and resolved electrophoretically. The numbers across the bottom of the gel denote consecutive sections beginning with the dermis and proceeding outward. Some sections were omitted, because they did not reveal significant changes. Tracks *1–7* were dermal, tracks *8–16* were basal and spinous layers, tracks *17–19* were granular layers and tracks *20–30* were str. corneum. To see more clearly the replacement of the 56 K and 58 K keratins by 55 K and 56.5 K keratins, tracks *9, 10, 17* and *20* are magnified in the *inset*. (Fuchs and Green 1980)

protein characteristic of fibroblasts (for review, see Lazarides 1982). The profiles in tracks 8 and 9 probably represent a mixture of keratins from basal cells and spinous cells. Since the dermal–epidermal junction is undulating, the basal epidermal layer was always contaminated to a degree with dermal fibroblasts on one side and cells from the spinous layer on the other. The keratins characteristic of rapidly growing cultured epidermal cells (46, 50, 56, and 58 K) were the most prominent bands in these sections, and probably represent the true basal keratins. Two of these keratins (46 and 50 K) belong to the type I keratin class and two (56 and 58 K) are type II keratins. The 67 K protein was most abundant in sections 14–16 and is most likely a type II spinous layer keratin (Kim et al. 1984). This keratin is not synthesized by the cultured keratinocytes and does not appear to be made in the basal epidermal cells. Other lines of evidence, including recent immunofluorescent studies using an antibody prepared against the 67 K keratin, have confirmed that the 67 K protein is exclusive to the suprabasal cells (Viac et al. 1980; Schlegel et al. 1980; Bowden and Cun-

liffe 1981; Woodcock-Mitchell et al. 1982; Warhol et al. 1983; Skerrow and Skerrow 1983).

Other changes in the pattern of keratins were also noted. Of particular interest is a keratin of 56.5 K that is synthesized in the spinous layers. This keratin is translated from a newly synthesized mRNA (Fuchs and Green 1980) and it also shows crossreactivity with antiserum prepared against the type I (50 K) keratin (Fig. 1). Thus, newly synthesized mRNAs encoding unusually large keratins of both the type I and the type II class seem to be produced in the differentiating cell.

The 63 K keratins, which are found only in palmar and plantar epidermis (Fuchs and Green 1980), also appeared to be synthesized in the spinous layers, but continued to accumulate in the granular and str. corneum layers. These keratins have not yet been classified according to type. A keratin of 65 K also appeared in the str. corneum, but it is presumably a breakdown product of the 67 K keratin.

It is interesting that the size of newly synthesized keratins of both types is increased during the course of terminal differentiation. Since the differentiation-specific changes involve both types of keratin, they may not disrupt the balance of the ratio of type I: type II keratins, and hence are unlikely to influence filament assembly per se. Nonetheless, the increase in the size of the two types of keratins may affect the properties and morphology of the keratin filament network inside the differentiating cell.

32.4 Changes in the Keratin Subunits During Embryonic Development of Skin

The epidermis of a 10-week human foetus consists of two layers: a basal layer and an upper periderm layer. By the 13th week of gestation, the first hair germ cells appear, and the epidermis consists of three layers. At the 20–40th weeks of development, additional layers are formed and hair follicles can be readily identified. Thereafter, the thickness of the epidermis increases somewhat and the str. corneum becomes more pronounced, and the differences between adult and foetal epidermis are less easily distinguished.

During the course of foetal development, the pattern of keratins produced by epidermal cells undergoes changes (Fig. 5). This was first demonstrated for lower mammalian species (Dale et al. 1976; Banks-Schlegel 1982) and later investigated in more detail for humans (Moll et al. 1982b; Holbrook et al. 1984). The only keratin which is present in all human embryonic stages is the 58 K keratin (Fig. 5, upper left; see also Moll et al. 1982b). In rabbit embryonic development, this keratin is not produced until day 18 of gestation when the epidermis becomes stratified (Banks-Schlegel 1982). On the basis of both this finding and also of the confined existence of the 58 K keratin in adult stratified squamous epithelia, it has been suggested that the 58 K keratin may be a marker for stratified squamous epithelia (Nelson and Sun 1982). Its presence in nonstratified human embryonic epidermis may indicate that the "destiny" of the developing epidermis to stratification is determined earlier in human than it is in lower mammalian species.
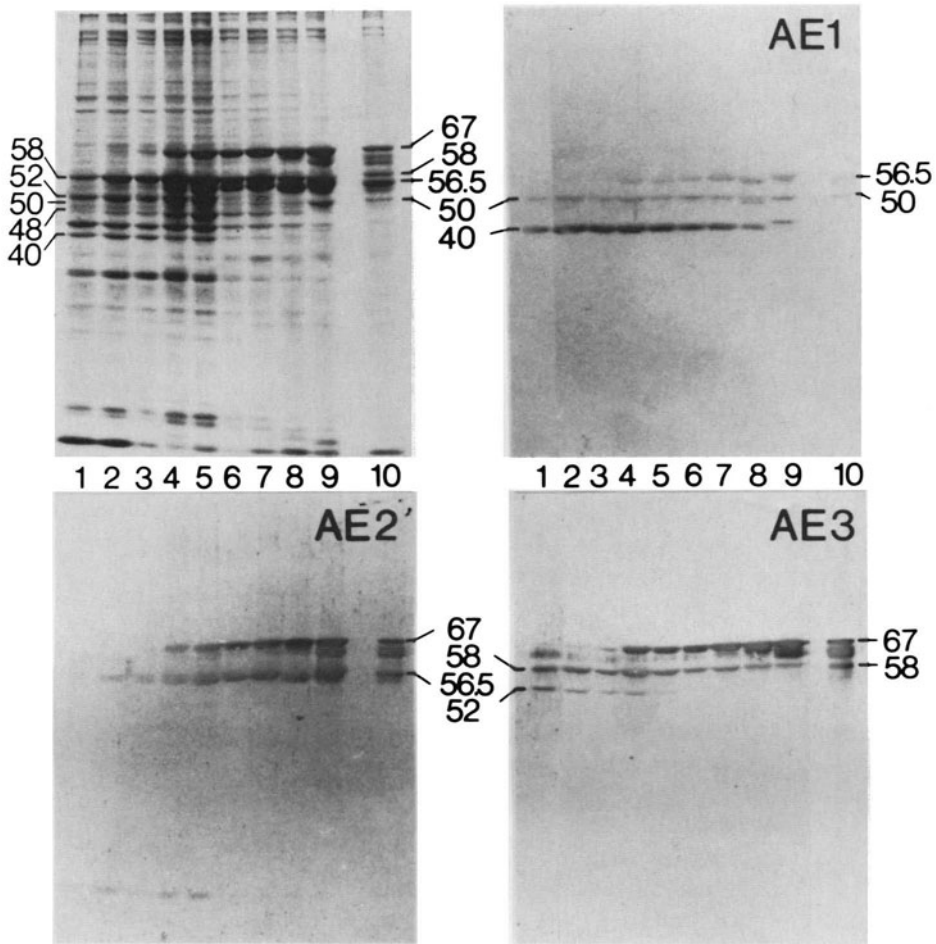
Fig. 5. Changes in the keratin subunits at different stages of development in human foetal epidermis. Epidermal proteins were separated by electrophoresis in SDS polyacrylamide gradient gels (7.5–15%). The gel stained with Coomassie blue is shown at the *upper left*. Proteins from duplicate gels were electrophoretically transferred to nitrocellulose and were subsequently identified by immunoblot analysis using AE1, AE2, and AE3 monoclonal antibodies to keratins (Woodcock-Mitchell et al. 1982). Lanes *1–8* foetal samples of estimated gestational age (EGA) 8, 9.5, 11, 12, 14, 16, 20, and 24 weeks, respectively; lane *9* newborn foreskin epidermis; lane *10* adult limb epidermis. The band at 43 K in the Coomassie-stained gel is probably actin. (Courtesy of Beverly Dale)

As judged by immunoblot analysis, the earliest keratins synthesized in foetal development are the 40 and 52 K keratins (Fig. 5, upper and lower right). These keratins begin to disappear by the 13th week of embryonic development (lane 5) and are virtually absent in normal adult epidermis (lane 10). It is interesting that these keratins are prominent in various "simple" adult epithelia (Franke et al. 1981a; Moll et al. 1982a; Tseng et al. 1982; Wu et al. 1982) and also in keratins of several squamous cell carcinoma lines of the epidermis and oral

epithelia (Wu and Rheinwald 1981). Perhaps even more remarkable is the fact that the synthesis of 40 and 52 K keratins can be reinduced in normal adult epidermal cells when cultured in vitro in the presence of tenfold higher than physiological concentrations of vitamin A (Fuchs and Green 1981). This observation leads to the attractive possibility that vitamin A may play an important role in regulating foetal development.

In contrast to the behavior of the "foetal" keratins, the 67 K keratin characteristic of terminally differentiating epidermis begins to appear at the 12th week and becomes a prominent component of adult epidermis (Fig. 5, lower left). Surprisingly, the first appearance of the 67 K keratin is confined to the intermediate layer of the embryonic epidermis, whereas all of the suprabasal layers of the adult epidermis contain the 67 K keratin or a slightly processed version of it (Moll et al. 1982b). For the most part, however, the temporal expression of the large keratins during development as well as differentiation confirms that these proteins are intimately related to the commitment of an epidermal cell to undergo terminal differentiation and str. corneum formation.

32.5 Regulation of Differential Keratin Gene Expression

How do the changes in keratin pattern that occur early during the course of terminal differentiation and embryonic development of the epidermal cell arise? Most studies indicate that the early changes in terminal differentiation involve alterations in the synthesis of the mRNAs for these proteins rather than major differences in posttranslational modifications of a single polypeptide chain (Fuchs and Green 1979, 1980; Schweizer and Goerttler 1980; Bladon et al. 1982; Roop et al. 1983). Thus, as the epidermal cell ceases to divide and undergoes a commitment to terminal differentiation, it still maintains its capacity to alter the transcriptional expression of its genes. Messenger RNA synthesis continues in the absence of DNA synthesis. The major change in keratin mRNA synthesis that occurs as the cell begins to differentiate is a switch to the synthesis of the mRNAs encoding the 67 K (type II) and 56.5 K (type I) keratins.

Once a cell passes through the spinous layer of the epidermis and enters the stratum granulosum, its biosynthetic activity ceases and hydrolytic processes become extremely active (Lavker and Matoltsy 1970). Thus, it is not surprising that the later appearance of the 65 and 55 K keratins of the str. corneum do not seem to arise from the translation of newly synthesized mRNAs, but rather from a post-translational processing mechanism (Fuchs and Green 1980; Bladon et al. 1982). Since as judged by one-dimensional peptide mapping, the 65 K keratin is similar to the 67 K keratin and the 55 K keratin is similar to the 56–58 K keratins, it is likely that limited proteolytic reduction in the size of the keratins takes place at this time (Fuchs and Green 1980).

Although the changes in the expression of keratin mRNAs during embryonic development has not yet been investigated, it is predicted that the observed changes in keratin patterns also reflect the appearance of new keratin mRNAs at different developmental stages. In studies involving cultured epidermal cells and cells from a squamous cell carcinoma of the tongue, mRNAs have now

been identified for most if not all of the keratins that are also made during human embryonic development (Fuchs and Green 1979, 1980; Kim et al. 1983).

Once the mRNAs are produced they are translated shortly thereafter (Kreis et al. 1983; Kim et al. 1984). Since the cytoplasm of the cell is an ideal environment for the spontaneous assembly of the keratin filament, it has been assumed that once the subunits are made, the filaments will be assembled. To investigate whether there are any translational controls of this process, mRNAs enriched for the bovine epidermal keratins have been microinjected into an epithelial cell that expresses a different complement of keratin mRNAs (Fig. 6, Franke et al. 1984). The endogenous keratins were sufficiently different from those encoded by the microinjected mRNAs for the two sets of keratins to be easily distinguished by two different antikeratin antisera. Double immunolabelling demonstrated clearly that the bovine epidermal keratin mRNAs are not only translated in cells that are already producing other keratin mRNAs, but in addition, the newly synthesized foreign polypeptides are incorporated into the preexisting network of epithelial keratin filaments (compare frame a, foreign keratin filaments with frame b, endogenous keratin filaments). Thus, the rate-limiting step in the synthesis of keratin polypeptides seems to be the appearance of keratin mRNA in the cytoplasm. Whether there is an intracellular feedback mechanism controlling the level of keratin polypeptide synthesis has not yet been thoroughly investigated.

The ability of foreign keratins to be incorporated into the preexisting filamentous network suggests that all the keratin polypeptides expressed within a single cell may be assembled into a single type of 8 nm filament comprised of a heterogeneous mixture of subunits. If indeed these results apply to the assembly of 8 nm filaments in vivo, then it is likely that the crucial step in changing the properties of the resulting keratin filaments resides in temporal changes in the expression of keratin mRNAs during different stages of differentiation and development.

To determine whether there may be any auxiliary proteins or factors that are necessary for filament assembly, mRNAs enriched for the bovine epidermal keratins were microinjected into a nonepithelial cell that does not produce any keratin filaments of its own (Fig. 7). In recipient cells, keratin mRNAs were translated in vivo and the resulting keratin subunits were subsequently assembled into 8 nm filaments. Double immunolabelling (not shown here) demonstrated clearly that the newly assembled keratin did not codistribute with microfilaments, microtubules, or the closely related vimentin intermediate filaments.

Whether keratin mRNAs can be translated and filaments can be assembled in the absence of any essential epithelial cell-specific components cannot be resolved with certainty, because the injected mRNAs were not pure keratin mRNAs. However, even when total poly (A) + RNA was injected into the foreign cell, the keratin filament network seemed very different from that visualized in epithelial cells. This was most likely due to the lack of desmosomes in the injected cell, which therefore did not permit the threading of the keratin filaments through these structures. Additional epithelial-specific filament-interacting proteins or factors may have also been absent in the nonepithelial cells. Although such components have not yet been identified, they may be important
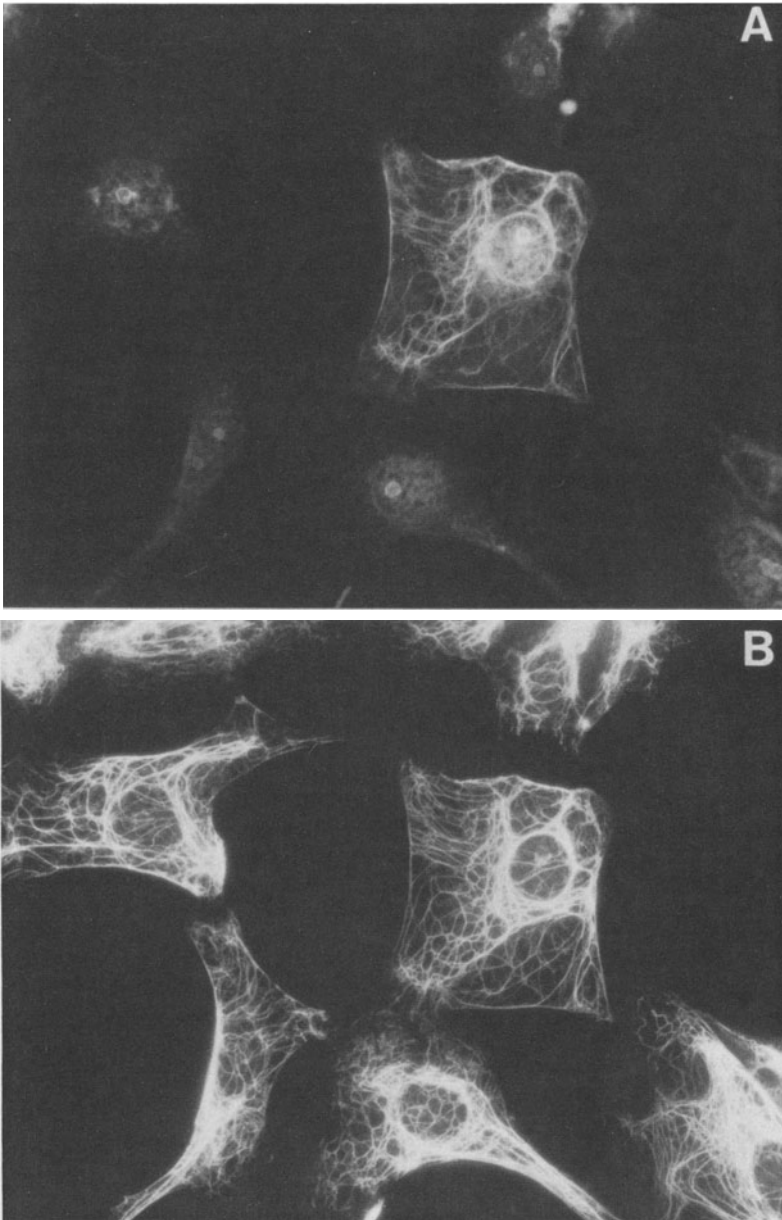
Fig. 6. Double immunofluorescence microscopy showing **A** a kangaroo rat kidney epithelial cell (PtK$_2$) microinjected with bovine epidermal keratin mRNA and stained with antibodies specific for epidermal keratins, and **B** the same cells stained with antibodies specific for the endogenous kidneytype keratins. Cells were stained 3 h after microinjection of mRNAs. Specific antibody binding was visualized using goat anti-rabbit IgG serum coupled to FITC(A) or rhodamine(B). Antibodies specific for either epidermal keratins (**A**) or endogenous kidney-type keratins (**B**) have been described (Kreis et al. 1983; Franke et al. 1984). Note that in **A**, only the injected cell contains keratins that are immunoreactive with the antiserum against epidermal keratins. (Courtesy of Werner W. Franke)
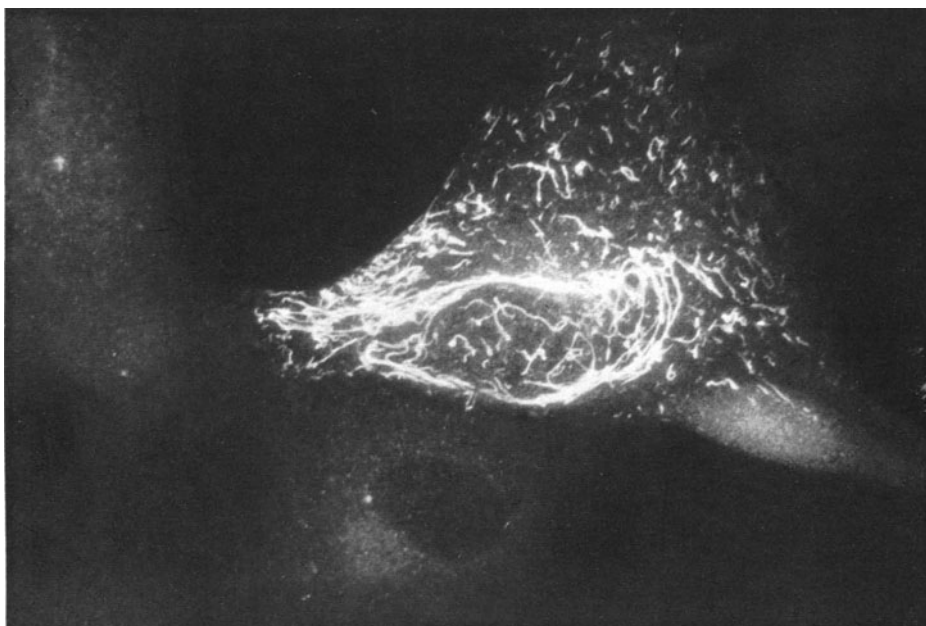
Fig. 7. Keratin filament assembly in a calf lens cell microinjected with bovine epidermal keratin mRNA. Cells were fixed at 16 h after microinjection. Keratins synthesized in vivo from the injected RNAs were visualized by indirect immunofluorescence microscopy using an antibody specific for epidermal keratins. Note that keratin filament assembly occurs de novo only in the injected cell, and that the pattern of filaments within the cell seems scattered, presumably due to the absence of desmosomes and desmosomal-filament interactions in the foreign cells. × 820. Courtesy of Werner W. Franke)

nonetheless in forming the proper cytoskeletal keratin filament architecture. Whatever the essential components, they did not seem to be produced by translation of microinjected poly (A) + epidermal RNAs. In the future, as these experiments and others are refined, identification and purification of proteins that interact with and influence the keratin filament network will undoubtedly become an important focal point for research.

32.6   The Role of Extracellular Factors in Regulating Terminal Differentiation and Keratin Gene Expression

The processes of terminal differentiation and development in epidermis are complex and seem to be controlled by a number of different factors. One of the major influential regulators is the fat-soluble isoprenoid, vitamin A. The vitamin has an antikeratinizing effect on epithelial differentiation such that when the level of the vitamin is reduced, the histological features characteristic of epidermal terminal differentiation become prominent (Fell and Mellanby 1953; Wolbach et al. 1954). Many aspects of epidermal behavior are also known to be

influenced by interaction with the dermis (Van Scott and Reinertson 1961; Sengel 1964; Billingham and Silvers 1968). Still other factors, including cAMP effectors (Green 1978), steroid hormones (Rheinwald and Green 1975) and epidermal growth factor (Rheinwald and Green 1977) have profound effects on the growth, if not also the differentiation, of the epidermal keratinocytes.

It has been shown that most of the effects of environmental factors on terminal differentiation can be studied using human epidermal cells cultured in vitro. These include stratification (Fusenig et al. 1981; Rheinwald and Green 1975; Hawley-Nelson 1980; Fuchs and Green 1981), mesenchymal–epidermal interactions (Rheinwald and Green 1975) and vitamin A (Fuchs and Green 1981). Mouse epidermal cells have also been cultured in vitro under conditions such that many of the differentiative functions of the cells are retained (Fusenig et al. 1975; Steinert and Yuspa 1978; Hennings et al. 1980, 1981). These cultures provide a very important experimental model to begin to investigate the biochemical mechanisms of regulation underlying the morphological alterations that take place during the course of terminal differentiation and embryonic development.

In light of the many changes in keratin patterns that occur during differentiation and development, it is not surprising that extracellular factors influence the expression of keratin genes. It has now been demonstrated that vitamin A in particular affects differentially the levels of certain keratin mRNAs (Fuchs and Green 1981; Kim et al. 1984). However, the exact nature of the roles played by these extracellular factors in regulating the expression of the 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 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 understanding the biochemical complexities and regulation of a family of distinct, but related proteins that can all assemble into filaments with similar structures but with quite different properties.

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