Prekeratin biosynthesis in human scalp epidermis

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Analysis of human scalp epidermal prekeratin polypeptides by two-dimensional gel electrophoresis revealed that each of the bands observed in one-dimensional electrophoresis consisted of three to five polypeptides of the same molecular weight but differing in isoelectric points. It was possible to divide the polypeptides into two families, with isoelectric points in the ranges pH 6.0–8.0 and pH 5.0–5.5 respectively. Incorporation of radiolabelled amino acids into freshly excised pieces of scalp epidermis showed that some of the polypeptides had relatively greater contents of glycine and serine than others. Radiolabelled methionine and leucine were, in contrast, incorporated more or less uniformly into all the polypeptides. After incubation with ^{32}P -labelled orthophosphate, relatively more intense labelling by ^{32}P was observed in the higher-molecular-weight bands of each family. The most basic of the isoelectric variants in each case did not take up phosphate, implying that at least some of the variation in charge was due to different degrees of phosphorylation. Polyadenylated RNA isolated from scalp epidermis was translated in an RNA-dependent reticulocyte haemolysate system followed by immunoprecipitation and electrophoresis. The polypeptides isolated by using anti-(human scalp prekeratin) immunoglobulin G had similar electrophoretic mobilities in sodium dodecyl sulphate/polyacrylamide gels to authentic prekeratin polypeptides, but had different isoelectric properties. This suggested that the products of keratin gene expression undergo post-translational modification.

Mammalian epidermis presents a tough but flexible barrier to the environment, but this barrier is provided largely by the layer of dead cells, or stratum corneum, forming the outer layers of the skin. The dead cells originate from the basal layer of the epidermis, the cells of which proliferate and migrate outwards towards the surface. As they do so they lose the ability to divide and commence the process of terminal differentiation. In the spinous and granular layers the cells continue to be active biosynthetically, at least for a time, but they then enter a destructive phase in which all the cellular organelles are eliminated. Eventually stratum corneum cells (corneocytes) are formed, which are cross-linked protein envelopes surrounding a deposit of keratin filaments: these cells have little or no metabolic activity.

Abbreviations used: SDS, sodium dodecyl sulphate; poly(A)⁺ RNA, polyadenylated RNA.

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Keratins may be extracted from epidermis by using buffers containing both protein-denaturing and disulphide-reducing reagents (Steinert & Idler, 1979; Fuchs & Green, 1980; Bowden & Cunliffe, 1981), and analysis of this material by SDS/polyacrylamide-gradient-gel electrophoresis reveals that it consists of a number of polypeptides. The use of milder extraction procedures (e.g. citric acid/sodium citrate buffer, pH 2.65; see Matoltsy, 1964; Skerrow, 1977) extracts from epidermis a material called prekeratin, which represents the precursor of mature keratin. This material originates from the lower layers (basal, spinous, granular) of the epidermis and it is also composed of a number of polypeptides. However, the molecular-weight distribution and relative abundance of these polypeptides differs from that of mature keratin (Fuchs & Green, 1980; Bowden & Cunliffe, 1981).

The process of terminal differentiation during keratinization therefore involves the conversion of prekeratin into keratin. This conversion includes cross-linking of the polypeptides, small decreases

in size of the polypeptides, a change in their isoelectric points, and perhaps other post-translational modifications, such as phosphorylation. However, this is not the whole story, as there are substantial changes in the pattern of keratin polypeptides synthesized, owing to changes in the spectrum of specific keratin mRNA molecules expressed at different times before the cells die (Fuchs & Green, 1978, 1980).

At present it is uncertain how many polypeptides constitute keratin or prekeratin. Attempts to separate the various components preparatively have had only limited success, and few amino acid analyses for individual chains have been reported (Steinert & Idler, 1975; Steinert *et al.*, 1980). There are, however, a number of amino acid analyses of total prekeratin and keratin in the literature (Skerrow, 1977; Baynes *et al.*, 1978). These show that the proteins have a relatively low content of cysteine residues (by comparison with the hard nail and hair keratins), and a relatively high content of glycine and serine residues. Hunter & Skerrow (1982) more recently compared the amino acid composition of prekeratin from normal human epidermis with that from involved psoriatic epidermis, which characteristically contains a lower amount of polypeptide of mol.wt. 70000. From their analyses they concluded that this polypeptide had a considerably higher content of glycine and serine than did the other tonofilament chains.

In the present work we have studied the incorporation of radioactively labelled glycine, serine, methionine, leucine and phosphate into the prekeratin of human scalp epidermis and have analysed the labelled polypeptides by one- and two-dimensional gel electrophoresis. We show that glycine and serine are preferentially incorporated into certain of the polypeptides and that phosphorylation accounts for at least some of the multiple isoelectric variants observed. A preliminary report of some of this work has appeared (Bladon *et al.*, 1982).

Materials and methods

Human scalp skin was obtained from a local hair-transplant clinic from subjects having no history of skin disorders. All samples were taken with the donors' consent and were used within 1 h of being removed.

Proteins used as molecular-weight markers were purchased from Sigma Chemical Co., Poole, Dorset, U.K., and Ampholines were purchased from LKB Instruments, Selsdon, Surrey CR2 9YD, U.K. SDS, specially purified for electrophoresis, was obtained from Pierce Chemical Co., Rockford, IL, U.S.A., and specially pure acrylamide, bisacrylamide and all other reagents were obtained from BDH Chemicals, Poole, Dorset, U.K. Radiochemicals were obtained

from Amersham International, and oligo(dT)-cellulose was from Collaborative Research, Waltham, MA, U.S.A.

Incorporation experiments in vitro

Skin samples were rinsed in distilled water and the epidermis was removed by a single stroke of an electrokeratotome set to cut at 0.1 mm. Samples were taken for histological examination to confirm that the skin was normal and also to assess the degree of dermal contamination (usually 8–10%).

Slices of scalp epidermis were incubated for up to 5 h at 37°C in Eagle's minimal essential medium supplemented with penicillin and streptomycin. To the incubation medium was added one or more of the following: [U-¹⁴C]glycine (113 mCi/mmol), L-[U-¹⁴C]serine (170 mCi/mmol), L-[4,5-³H]leucine (65 Ci/mmol), L-[³⁵S]methionine (>1000 Ci/mmol) or [³²P]orthophosphate (98% ³²P, 10 mCi/ml), as described by Bladon *et al.* (1982).

After incubation the pieces of epidermis were rinsed in fresh ice-cold medium without labelled compound, and the prekeratin was extracted with 0.1 M-citric acid/trisodium citrate buffer, pH 2.65, exactly as described previously (Bowden & Cunliffe, 1981).

Electrophoretic methods

Prekeratin samples were analysed by single-dimension SDS/polyacrylamide slab gradient gels as described previously (Bowden & Cunliffe, 1981). Analysis by the two-dimensional method (isoelectric focusing followed by SDS/polyacrylamide-gel electrophoresis) was performed essentially as described by Ames & Nikaido (1976) with a Bio-Rad Protean Cell apparatus. Gels were stained with Coomassie Blue R to show the protein bands and were dried, either with or without fluorography, before exposure to Fuji RX safety film to detect radioactivity. In some instances the film was pre-flashed (Laskey & Mills, 1975).

Phosphorylated amino acids were detected by high-voltage paper electrophoresis of hydrolysed ³²P-labelled prekeratin. The hydrolysis was performed for 2 h at 100°C in 6 M-HCl, after which the sample was evaporated to dryness. This sample was dissolved in water, spotted on to Whatman 3MM paper and electrophoresed in acetic acid/formic acid/water (2:1:25, by vol.). Marker solutions of authentic phosphoserine and phosphothreonine were run simultaneously. The electrophoretogram was air-dried, washed in 3% (v/v) triethylamine, dried again, and developed by dripping in a solution of fluorescamine in triethylamine made by diluting a stock 1 mg/ml acetone solution of the fluorescamine 1:10. Autoradiographic analysis of the dried electrophoretogram was performed against Kodak XRP film for 5–10 days.

Amino acid analysis

Samples of prekeratin were hydrolysed with 6M-HCl in sealed, evacuated tubes for 24–96 h at 110°C, and the amino acid content was analysed with a Chromaspek J-180 instrument. Values for serine and threonine were corrected by extrapolating to zero time. The values for valine, isoleucine and histidine are those obtained after 96 h of hydrolysis. Cysteine was determined as cysteic acid. The values for all the other amino acids are averages of the values obtained after different times of hydrolysis. Tryptophan was not determined.

Extraction and translation of poly(A)⁺ RNA

Sterilized solutions and equipment were used throughout. Total RNA was isolated from scalp epidermis by the LiCl/urea/phenol/chloroform method (Applebaum *et al.*, 1981), and poly(A)⁺-containing RNA was isolated from this by chromatography on oligo(dT)-cellulose. The total RNA was dissolved in 0.5% (w/v) SDS/0.5M-NaCl/1 mM-EDTA/10mM-Tris/HCl buffer, pH 7.4, and applied to the column, which was equilibrated in the same buffer. After washing through with the same buffer lacking SDS, the column temperature was raised to 60°C and the poly(A)⁺ RNA was eluted with 10mM-Tris/HCl buffer, pH 7.4, precipitated with ethanol at -20°C overnight and recovered by centrifugation. The yield of poly(A)⁺ RNA from 2–4 g of epidermis was 20–30 µg. The poly(A)⁺ RNA so obtained was translated in the micrococcal-nuclease-treated rabbit reticulocyte haemolysate system in the presence of [³⁵S]methionine for 1 h at 30°C (Pelham & Jackson, 1976).

Analysis of translation products

Antibodies were raised in rabbits by injecting prekeratin dissolved in 0.05M-Tris/HCl buffer, pH 7.2, containing 6M-urea, and the immunoglobulin fraction was purified as described by Harboe & Inghild (1973). The translation products were treated with the immunoglobulin G and the immunoprecipitate was analysed by gel electrophoresis as described by Wood & Bonaventura (1981).

Results

Characterization of prekeratin polypeptides

Human epidermal prekeratin consisted of four major groups of polypeptides (A–D) when separated on the basis of size by SDS/polyacrylamide-gel electrophoresis. The molecular-weight range of the polypeptides was 50 000–70 000, and the values of the major bands are shown in Fig. 1(a). The fifth band observed (E), with a mol.wt. of about 46 000, is not thought to be a prekeratin polypeptide, but may be actin. Two-dimensional electrophoresis (Fig. 1b) revealed that each of the 'bands' A–D was

in fact a group of polypeptides having a range of isoelectric points. We have designated these by a lower-case letter indicating their origin, and a subscript number, the isoelectric variants being enumerated starting at the 'neutral' end of the range; hence band A gives rise to the isoelectric variants a₁, a₂, a₃ etc.

Fig. 1. (a) SDS/polyacrylamide-gradient slab gel electrophoresis, and (b) two-dimensional electrophoresis, with human scalp epidermal prekeratin

(a) The gradient of polyacrylamide was 7.5–17.5% and the gel was stained with Coomassie Blue R. The values show the molecular weights ($\times 10^{-3}$) of the bands based on calibration with marker proteins of known molecular weights. (b) Isoelectric focusing (IEF) in the first dimension over the pH range shown followed by SDS/polyacrylamide-gel electrophoresis in the second dimension on a 7.5–17.5% gradient slab gel. On the left is the same material applied in the second dimension only. In both cases of SDS/polyacrylamide-gel electrophoresis the positive electrode is at the bottom.

Fig. 2. Incorporation of radiolabelled amino acids into human scalp prekeratin polypeptides
(a) Stained gel (conditions as in Fig. 1a legend), showing that the material extracted has the normal polypeptide pattern; standard proteins (Stds) and

The higher-molecular-weight polypeptides comprising bands A and B were shown to be composed of families of polypeptides with near-neutral isoelectric points (Fig. 1b). The lower-molecular-weight polypeptides from bands C and D, in contrast, were composed of families of polypeptides with relatively acid isoelectric points. Typically three to five variants were observed from each of the bands A–D. Band E occupies approximately the position on the two-dimensional gel expected of actin.

Amino acid analysis (Table 1) showed that human epidermal prekeratin as extracted by our procedure was especially abundant in the residues glycine and serine.

Prekeratin biosynthesis in pieces of epidermis

Incorporation experiments *in vitro* showed that labelled amino acids were incorporated into prekeratin by pieces of human epidermis freshly removed from patients. Fig. 2(a) shows the Coomassie Blue-stained gel and Fig. 2(b) shows the corresponding autoradiograph. The amino acids glycine and serine were preferentially incorporated into the

Table 1. Amino acid analysis of human scalp epidermal prekeratin

Results are expressed as residues per 100 residues. Appropriate corrections were made for the destruction and slow release of residues. Tryptophan was not determined.

Amino acid	Content
Aspartic acid	10.12
Threonine	4.57
Serine	8.81
Glutamic acid	14.38
Glycine	16.96
Alanine	6.16
Valine	5.29
Isoleucine	4.57
Leucine	8.33
Tyrosine	4.77
Phenylalanine	3.28
Histidine	2.79
Lysine	4.96
Arginine	3.58
Proline	1.42
Cysteic acid	0.45
Methionine	<1

molecular-weight values ($\times 10^{-3}$) are shown on the right. The values on the left are the molecular weights ($\times 10^{-3}$) of the prekeratin polypeptides. Gly, Ser and Met refer to the isotopically labelled amino acid used in the incorporation, i.e. [^{14}C]glycine, [^{14}C]serine or [^{35}S]methionine. (b) Corresponding autoradiograph after incorporation of [^{14}C]glycine, [^{14}C]serine or [^{35}S]methionine.

Fig. 3. Two-dimensional gel electrophoresis with radio-labelled prekeratin polypeptides

Prekeratin was prepared from scalp epidermis that had been incubated with a radiolabelled amino acid and was subjected to two-dimensional electrophoresis as described in the legend to Fig. 1b, with which these patterns should be compared; (a) fluorograph of [^{14}C]glycine incorporation; (b)

heavier polypeptides and especially into the 70000- and 57000-mol.wt. bands. In contrast, methionine incorporation appeared to be similar to all of the bands (Fig. 2b), with perhaps slightly more intense labelling to the 52000-mol.wt. band. In an experiment in which the time course of the incorporation of [^{35}S]methionine into prekeratin was followed over 1 h, it appeared that all the polypeptides were biosynthesized at about the same rate, as judged by the relative intensities of the bands. It is possible that methionine has a differential rate of incorporation into the polypeptides which more or less balanced their different methionine contents (although we do not at present have amino acid analyses of the different bands). However, experiments in which the incorporation of [^3H]leucine was followed (results not shown) also revealed a more-or-less even labelling of all the polypeptides, suggesting that this is not the case.

An examination of the glycine-, serine- and methionine-labelled prekeratin by two-dimensional electrophoresis (Fig. 3) revealed that glycine and serine had been incorporated preferentially into the higher-molecular-weight chains of each family of polypeptides, i.e. A and C, and that all of the isoelectric variants had taken up label. Methionine, in contrast, labelled all of the polypeptides to approximately equal intensities, with perhaps slightly more intense labelling of the lower-molecular-weight bands. On the basis that the mixture of polypeptides forming prekeratin as extracted is relatively rich in glycine and serine residues (Table 1), our results seem to show that certain of the heavier polypeptides are even richer in these residues.

Phosphorylation of prekeratin

Incorporation of [^{32}P]orthophosphate occurred preferentially into the higher-molecular-weight polypeptides of each family (i.e. A and C), producing a somewhat similar pattern of labelling in one-dimensional electrophoresis to that observed for glycine and serine incorporation. Because of the higher energy of the radioactive phosphorus, the bands were less clearly defined (Fig. 4a). Analysis of the synthesized polypeptides by two-dimensional electrophoresis showed that all four major groups (A–D) were in fact phosphorylated. All but the most basic of the isoelectric variants appeared to be phos-

fluorograph of [^{14}C]serine incorporation; (c) fluorograph of [^{35}S]methionine incorporation. The upper-case letters on the left refer to the bands as separated by single-dimension SDS/polyacrylamide-gel electrophoresis (see Fig. 1a), and the numbers represent their molecular weights ($\times 10^{-3}$) as measured by calibration markers. The lower-case letters identify the families of isoelectric variants.

phorylated, e.g., b_2 - b_5 but not b_1 (Fig. 4b). Polypeptide E did not take up any significant amount of ^{32}P .

Hydrolysis of total extracted prekeratin with 6M-HCl for 2 h at 100°C *in vacuo* followed by high-voltage paper electrophoresis and autoradiography revealed the presence of a single spot on the auto-

Fig. 4. Incorporation of ^{32}P into prekeratin polypeptides. Electrophoresis in (a) one and (b) two dimensions as was described in the legend to Fig. 1. Pieces of scalp epidermis were incubated in the presence of ^{32}P -orthophosphate and the prekeratin was extracted and subjected to electrophoresis followed by autoradiography. The two-dimensional pattern should be compared with that shown in Fig. 1(b).

Fig. 5. RNA-directed biosynthesis of keratin gene products.

Poly(A)⁺ mRNA was isolated from human scalp epidermis and translated in the rabbit reticulocyte-haemolysate system in the presence of ^{35}S -methionine. (a) (i) Polypeptides isolated from the total translation products by using anti-(scalp prekeratin) immunoglobulin G, in comparison with (ii) the spectrum of prekeratin polypeptides isolated from scalp epidermis incubated with ^{35}S -methionine. The upper-case letters identify the bands and the numbers refer to their molecular weights ($\times 10^{-3}$) as in Fig. 1(a). (b) Two-dimensional gel (conditions as in Fig. 1b legend) run with the total translation products. The keratin-gene products (c and d) were identified by comparison with authentic unlabelled prekeratin run on the same two-dimensional gel. Polypeptides a and b are presumed not to be observed because they have higher isoelectric points than pH 7.2 before phosphorylation (which does not occur in the reticulocyte-lysate system).

radiogram which moved with the same mobility as authentic *O*-phosphoserine. *O*-Phosphothreonine and *O*-phosphotyrosine were not detected among the hydrolysis products.

Cell-free biosynthesis of prekeratin

Isolated poly(A)⁺ RNA from human epidermis directed the cell-free synthesis of polypeptides in the reticulocyte haemolysate system. The yield of poly(A)⁺ RNA was poor (e.g. less than 20 µg/g fresh wt. of tissue), and high incorporation of [³⁵S]-methionine into synthesized polypeptides was not achieved reproducibly (Table 2). This is attributed to the presence of large amounts of ribonuclease in epidermis and is the experience of other workers with skin mRNA (Schweizer & Goerttler, 1980).

Analysis of the total translation products by SDS/polyacrylamide-gel electrophoresis showed that a range of polypeptides was being synthesized, and it was not possible to say that the translation products were especially rich in polypeptides in the prekeratin region (i.e. mol.wts. 40 000–70 000). Immunoprecipitation with rabbit anti-prekeratin immunoglobulin G isolated about five polypeptides with approximately the same size-distribution as is found in mature prekeratin or in prekeratin labelled *in vitro* by incubation of pieces of epidermis with [³⁵S]methionine. The comparison is shown in Fig. 5(a). If it is recalled that the polypeptides synthesized in this latter way are also phosphorylated, it may be concluded that the extent of phosphorylation is not so great as to cause changes in the extent of SDS binding and thus mobility of the polypeptides in SDS/polyacrylamide-gel electrophoresis.

The total translation products were examined by two-dimensional gel electrophoresis (Fig. 5b). Unlabelled prekeratin was added before electrophoresis so that the prekeratin polypeptides could be identified in the stained gel. Fig. 5(b) shows the corresponding autoradiograph. At least three ‘acid’ polypeptides were translated (e.g. c₁⁺, c₁, d₁), but few if any isoelectric variants were observed to carry [³⁵S]-methionine. We interpret this to mean that the initial products of translation are comparatively small in

number and that subsequent phosphorylation, which does not seem to occur to any great extent in the reticulocyte haemolysate system, results in the production of multiple isoelectric variants.

Discussion

Many early studies were done in which the incorporation of radiolabelled amino acids into epidermal proteins was followed (reviewed by Bernstein *et al.*, 1970), and the histidine-rich protein (see Scott & Harding, 1981) was identified as a result of such studies. However, analyses of the labelled protein were limited by the lack of high-resolution electrophoresis systems.

The use of the SDS/polyacrylamide-gradient system gives extremely good resolution of the polypeptides as a function of size (Bowden & Cunliffe, 1981) and we have now extended this analysis to investigate isoelectric variants by using the two-dimensional gel system. On the basis of this, families of polypeptides have been identified. Sun & Green (1978) analysed human (heel) callus keratin in the O’Farrell (1975) two-dimensional gel system and compared their results with those obtained with keratin from cultured cells. Gilmartin *et al.* (1980) also used two-dimensional gels to investigate the keratin of human epidermis. Both groups examined material which had been extracted from epidermis with urea and mercaptoethanol, or SDS and mercaptoethanol, usually after a neutral low-ionic-strength Tris wash. The material they extracted would therefore be a mixture of keratin and prekeratin. In the present work we have studied prekeratin that had been extracted with 0.1M-citric acid/sodium citrate buffer, pH 2.65. It is therefore not surprising that the two-dimensional gels show an overall similarity in the distribution of polypeptides, but differ in detail (see Fig. 3). We have in fact observed a transition from the prekeratin polypeptide pattern to the keratin polypeptide pattern (Bowden *et al.*, 1981). However, it should be noted that during the time-scale of the incorporation experiments described in the present paper, only the prekeratin polypeptides became labelled.

Table 2. Incorporation of [³⁵S]methionine into trichloroacetic acid-precipitable translation products by micrococcal nuclease-treated rabbit reticulocyte haemolysate

The poly(A)⁺ RNA was added to a concentration of 50 µg/ml in each case. The amino acid mixture in the haemolysate was that appropriate for prekeratin (see Table 1).

Method of preparation of total RNA	Number of passages through oligo(dT)–cellulose	Acid-precipitable radioactivity (c.p.m.) (minus background) in 5 µl translation mixture
Phenol/chloroform	1	16 017
Phenol/chloroform	2	41 484
Guanidinium chloride	1	19 513

We divided the prekeratin polypeptides into families based on their behaviour in gel electrophoresis. It is of interest in this respect that Fuchs *et al.* (1981), on the basis of cloning experiments, identified two subfamilies of keratin genes with about ten genes per family. One class was the polypeptides of mol.wt. 56 000–58 000, and the other those of mol.wt. 46 000–50 000. All the vertebrates that they examined using the probes that they had prepared were found to contain discrete sequences homologous to both of the human keratin classes. These cloning experiments were performed starting with mRNA isolated from cultured human epidermal cells. These of course do not make the 70 000-mol.wt. polypeptide found in human prekeratin (Sun & Green, 1978) and which is suggested by Hunter & Skerrow (1982) to be rich in glycine and serine residues. Our work seems to confirm that the 70 000-mol.wt. polypeptide is indeed glycine- and serine-rich and should in fact have a higher glycine and serine content than that of total prekeratin polypeptides (Table 1). This polypeptide is known to be present in lower amounts in prekeratin taken from the lesions of the human hyperkeratinizing condition psoriasis (Skerrow & Hunter, 1978; P. E. Bowden, E. J. Wood & W. J. Cunliffe, unpublished work). This disease is believed to have a genetic origin, and it will be of great interest to see which family of genes the 70 000-mol.wt. polypeptide belongs.

Gilmartin *et al.* (1980) studied the incorporation of [^{32}P]orthophosphate into rat epidermis both *in vitro* and *in vivo*, into cultured human cells and into human punch-biopsy samples. After incubation with the radioisotope, the keratins were extracted with SDS in the presence of 2-mercaptoethanol, and it was shown by gel electrophoresis and autoradiography that the polypeptides had taken up phosphate. Three major ^{32}P -containing bands were identified on the gels of keratin from cultured human cells, which corresponded to mol.wts. of 44 000, 54 000 and 55 000. These are the major bands observed in the electrophoresis system used by Gilmartin *et al.* (1980). They also reported that a higher-molecular-weight phosphorylated polypeptide (63 000) was present in keratin from normal human epidermis, but was not found in keratin from psoriatic epidermis, as would be expected (see above). Our results show that for normal human prekeratin, the major bands (really groups of bands) corresponding to mol.wts. of 52 000, 57 000 and 70 000 are phosphorylated, and it is likely that the multiple isoelectric variants observed in two-dimensional gel electrophoresis arise, at least in part, from different degrees of phosphorylation. Our results also indicate that phosphorylation takes place very early in the formation of epidermal keratins, probably soon after the polypeptides are synthesized.

Steinert *et al.* (1982) have presented an extensive

study of the phosphorylation of mouse and bovine keratins. They showed that the isolated subunits, K_1 and K_2 , of mouse keratin contained 4–5 mol. of *O*-phosphoserine/mol of protein and that only some of the subunits of bovine keratin were phosphorylated. Although they separated mouse keratins into two 'bands' by preparative gel electrophoresis, one analysis of mouse keratins by high-resolution gradient-gel electrophoresis (P. T. Bladon, M. Taylor, E. J. Wood & W. J. Cunliffe, unpublished work) suggested that each of these bands is likely to contain a number of components. Both Gilmartin *et al.* (1980) and Steinert *et al.* (1982) found with human keratins, as we did with human prekeratins, that serine was the residue phosphorylated. However, Steinert *et al.* (1982) reported that some keratin subunits (e.g. mouse K_1) contained small amounts of *O*-phosphothreonine (less than 1 mol/mol). Steinert *et al.* (1982) also showed that one of the bovine subunits could be dephosphorylated with alkaline phosphatase, the process passing through a number of stages of partial dephosphorylation to give finally one or two spots with an isoelectric point of pH 5.8, compared with an original of pH 5.2. The phosphate was shown to be associated with the non-helical regions of the polypeptides.

It remains to determine what role keratin phosphorylation plays. A possible role in filament assembly would seem to be ruled out by the observation that dephosphorylated subunits seem to be perfectly capable of participating in filament formation (Steinert *et al.*, 1982).

The mRNA for keratin has been identified and translated in a cell-free system by several groups of workers with several different species (Schweizer & Goerttler, 1980; Fuchs & Green, 1979, 1980; Gibbs & Freedberg, 1980). All groups seemed to experience difficulties in isolating mRNA, presumably because of high tissue ribonuclease activity. Probably in the upper layers of the epidermis, when cellular organelles are being eliminated, there is a considerable amount of lysosomal activity in the cells. Fuchs & Green (1980) concluded that the changes in keratin composition taking place during terminal differentiation of human keratin resulted largely from changes in synthesis. The polypeptides were shown to be translated from different mRNA molecules. Our results in general confirm these findings, and we have been able to identify some, but not all, of the components in two-dimensional gel electrophoresis (Fig. 5). Gibbs & Freedberg (1982), studying mRNA from guinea-pig skin, have identified other components with higher isoelectric points by using a much wider pH range on their two-dimensional gels (i.e. pH 3.5–10.0).

These findings strongly suggest that there are comparatively few products of translation, but that isoelectric variants arise as a result of phosphoryla-

tion. However, the occurrence of other post-translational modifications cannot be ruled out. Now that some of the keratin genes have been cloned (Fuchs *et al.*, 1981), we can look forward to a rapid increase in our understanding of the structure of the keratin genes and their expression.

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