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Stratification-related expression of isoforms of the desmosomal cadherins in human epidermis

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SUMMARY

Desmosomal junctions are abundant in epidermis and contain two classes of transmembrane glycoprotein, the desmocollins and the desmogleins, which are members of the cadherin superfamily of Ca^{2+}-dependent cell adhesion molecules. The desmocollin subfamily includes DGIV/V and DGII/III while the desmoglein subfamily includes DGI, HDGC and the autoantigen of the blistering skin disease pemphigus vulgaris (PVA). There are also several non-glycosylated proteins, including the desmoplakins and plakoglobin, present in the desmosomal plaque, which forms a link between the glycoproteins and the cytokeratin intermediate filaments. To provide a picture of the expression of the desmosomal genes and their products in epidermis, we have used *in situ* hybridisation and immunofluorescence staining on sections of human foreskin. We find that, as expected, desmoplakin DPI/II and plakoglobin are expressed throughout the epidermis, gradually accumulating during differentiation, which probably reflects the increased numbers of desmosomes. In contrast, while keratin 14 and the hemidesmosomal component bullous pemphigoid antigen I (BPAGI) are basal-specific, desmocollin DGIV/V is expressed only in the upper spinous/granular layers of the epidermis, whereas DGII/III expression is enriched in the basal layers. Amongst the desmogleins, expression of DGI appears similar to desmoplakin and plakoglobin; PVA is more prevalent in the lower spinous layers, whereas HDGC expression is detected basally but not suprabasally. The major desmosomal cadherin transcripts are desmocollin DGIV/V and desmoglein DGI. The resultant changes in desmosomal composition and structure may reflect the maturation of desmosomes, presumably being related to the need for changes in cell adhesion during stratification, terminal differentiation, and desquamation, and point to the desmosome being a key player in epidermal differentiation.

Key words: desmosomes, cadherin, epidermis

INTRODUCTION

Growth and differentiation of the epidermis is marked by changes in morphology and biochemical composition as keratinocytes migrate from the basal layer through the spinous and granular layers. Keratinisation culminates in the production of terminally differentiated cells in the stratum corneum, which are sloughed from the surface and continually replaced by differentiating cells from below. In particular, changes in the intermediate filaments of the epidermal cytoskeleton (cytokeratins) have been described as differentiation occurs (for review, see Fuchs, 1990). Thus basal cells have a cytoskeleton composed of a relatively dispersed, but extensive, network of cytokeratin filaments, which are made up of a 1:1 ratio of two keratin proteins, K5 and K14 (Nelson and Sun, 1983). Cells in the first suprabasal layer, the spinous layer, are larger than cells in the basal layer and synthesise two new keratins, K1 and K10, forming cytoskeletal filaments that aggregate into thin (tonofilament) bundles (Eichner et al., 1986).

Desmosomes are cell junctions which provide anchorage for intermediate filaments and thus interconnect the cells into a three-dimensional lattice (for reviews, see Franke et al., 1987; Garrod et al., 1990; Schwarz et al., 1990). Desmosomes are present in the basal layer, increase in number and change in dimensions and electron density before the final breakdown in the later stages of keratinization (White and Gohari, 1984; Skerrow et al., 1989; Chapman and Walsh, 1990). By the time the stratum corneum is reached, there is an impermeable and highly protective layer in which desmosomes appear to play little part in cell-cell adhesion.

Some tissue specificity has been described in the proteins of the urea-soluble desmosomal plaque; thus DPIV (‘band 6’) (Kapprell et al., 1988) and desmoyokin (Hieda et al., 1989) are found only in stratified epithelia. One report that

desmoplakin DPII, an alternatively spliced product (Green et al., 1990) of the same gene DSP (Arnemann et al., 1991) coding for desmoplakin DPI, was restricted to stratified epithelia (Cowin et al., 1985) has been challenged (Angst et al., 1990).

For some time there has been immunological evidence that there is heterogeneity in the composition of the glycosylated proteins, the desmocollins and desmogleins, which are present in the urea-insoluble desmosomal 'cores'. Immunological cross-reactivity of desmogleins and desmocollins among species and within tissues in the same species has been reported to differ (Giudice et al., 1984; Suhrbier and Garrod, 1986). Significantly, certain mouse polyclonal anti-desmocollin antisera (Parrish et al., 1986) and monoclonal anti-desmoglein antibodies (Jones et al., 1987) only recognise desmosomes from suprabasal cells.

Definite evidence for the stratification-related expression of isoforms of the desmosomal glycoproteins (King et al., 1991) has been found subsequent to the cloning and molecular characterisation of the cDNAs coding for these proteins. cDNAs of the desmocollins and desmogleins have been cloned from human (Nilles et al., 1991; Parker et al., 1991; Wheeler et al., 1991a, b) and bovine sources (Goodwin et al., 1990; Koch et al., 1990, 1992; Collins et al., 1991; Mechanic et al., 1991) (for reviews, see Magee and Buxton, 1991 and Buxton and Magee, 1992). Sequence analysis of these clones has shown that the desmosomal glycoproteins are members of the cadherin superfamily of cell-cell adhesion molecules, making them prime candidates for the role of cell adhesion within the desmosome. Comparison of the human and bovine desmocollin cDNA clones, together with amino acid analysis of desmocollin-like glycoproteins from human epidermis, led to the realisation that there were at least two isoforms of the desmocollins, with similar but different sequences, coded by distinct genes. Immunofluorescence experiments with monoclonal and polyclonal anti-desmocollin antibodies demonstrated that one desmocollin isoform, DGIV/V, was expressed only suprabasally (King et al., 1991). A monoclonal antibody recognising the other class, DGII/III, stained throughout the living layers of the epidermis, but DGIV/V and DGII/III may share epitopes, making it difficult to detect differences in gene expression within the epidermis solely by immunological means. A similar problem has been emphasised in the expression of the keratin genes (Bosch et al., 1988). It was therefore necessary to perform *in situ* hybridisation experiments to determine specific expression of these closely related genes. The initially isolated human desmocollin cDNA clone was of the DGII/III type (Parker et al., 1991), human cDNA clones of the DGIV/V type having been subsequently isolated (I.A. King, unpublished data), whereas the first bovine clones (Collins et al., 1991; Koch et al., 1991b; Mechanic et al., 1991) were of the DGIV/V type and expressed only suprabasally (Koch et al., 1991b), a bovine DGII/III type has recently been reported (Koch et al., 1992).

Recent cDNA cloning results have also identified two more desmoglein isoforms with stratification-related expression, in addition to the originally described DGI (gene DSG1). These are HDGC (Koch et al., 1991a) (encoded by gene DSG2) isolated from a simple epithelial human colon carcinoma cell line, and PVA (gene DSG3), the autoantigen DSG3 from the blistering skin disease pemphigus vulgaris (Amagai et al., 1991). Prior to its identification as a desmoglein, PVA was shown by immunofluorescence (Beutner et al., 1968) to be limited to stratified squamous epithelia, now confirmed by Northern blotting with a cDNA clone as probe (Amagai et al., 1991). PVA has not been definitely shown to be a desmosomal glycoprotein; it may be along the entire cell surface of the keratinocyte and not necessarily located exclusively in desmosomal junctions, although recent immunogold labelling studies suggest that it is indeed concentrated in desmosomes (J.R. Stanley, personal communication).

In this paper we present the results of *in situ* hybridisation experiments on human epidermis with antisense probes for the two desmocollins, DGIV/V and DGII/III, and the three desmogleins, DGI, HDGC and PVA, together with probes for the desmosome plaque proteins desmoplakins DPI/II (gene DSP) and plakoglobin DPIII (gene JUP), the basally expressed keratin K14 and bullous pemphigoid antigen I (gene BPAG1), a component of hemidesmosomes. The results demonstrate that the various isoforms of the desmosomal cadherins are expressed in a stratification-related manner, reminiscent of the keratin isoforms. In view of their potential as cell adhesion molecules, this suggests that the adhesive capacity of desmosomes changes as stratification proceeds.

MATERIALS AND METHODS

Reverse transcription-PCR (RT-PCR)

Total RNA was isolated from cultured keratinocytes by the guanidinium isothiocyanate extraction method (Chirgwin et al., 1979). Poly(A)$^+$ RNA was separated by standard methods using oligo(dT)-cellulose. In two parallel experiments 1 μg of poly(A) RNA was primed with oligo(dT) and hexanucleotide primers, and subsequently reverse transcribed using a commercially available cDNA synthesis kit (Boehringer Mannheim). About 1/10 of the volume was used in a direct PCR or nested PCR amplification reaction, as described earlier (Arnemann et al., 1991). In order to amplify the desired genes the following sequence-specific primers were used:

Desmoplakin : DPI/II (clone pJA44T4)

Nest I: JA98, 5′-TGGTAGATAGAAAGACAGGC-3′ (pos. 4199-4218); JA82, 5′-GTGCCCAATAGAACTACTGC-3′ (pos. 5256-5237).

Nest II: JA95B, 5′-GTAGGAAGAATTCCCTGTGGC-3′ (pos. 5217-5197); JA99, 5′-ACAAGGGCCTTGTTGTTGACAGG-3′ (pos. 4247-4267).

Bullous pemphigoid antigen I : BPAGI (clone pJA34T4)

Nest I: JA119, 5′-GCTTCTGGACATGAGAACAAT-3′ (pos. 4654-4674); JA120, 5′-GTGTTTCTTGACATTGAGATTGG-3′ (pos. 5344-5325).

Nest II: JA121, 5′-GAACAATGGATCCGCTGCGAC-3′ (pos. 4668-4688); JA122, 5′-GAGATTGGAAAGCTTCCTCTCC-3′ (pos. 5332-5311).

Keratin K14 (clone pJA80T2)

JA150, 5′-GCAAGGATCCCGAGGAATGG-3′ (pos. 3366-3385), JA151, 5′-TGAAGCTTTATTGATTGCCAGG-3′ (pos. 4863-4842).

Pemphigus vulgaris antigen: PVA (clone pJA85T2)

JA135, 5′-TTAGGAGTGACAACATATTGC-3′ (pos. 3271-3251); JA140, 5′-TGACCACTAAGCTTGGAGC-3′ (pos. 2245-2263).

Desmoglein HDGC (clone pJA77T2)

JA156, 5′-CAGTAGCTTCCCAGTTCC-3′ (pos. 324-341), JA157, 5′-CTGTAAGCTTCATGAAAAATCAG-3′ (pos. 1126-1104).

The PCR products were subsequently subcloned into Bluescript or pGEM-11Zf(−) vectors and their identity confirmed by sequencing using the Sequenase kit (United States Biochemical Corp.).

cDNA clones

Clone pHPG Ca2.1 coding for plakoglobin was a kind gift from W.W. Franke. Clone pJA40T10 is a *Bgl*II/*Eco*RI subclone of the cDNA clone pG4 coding for desmoglein DGI (Wheeler et al., 1991b). Clone pJA2D8 is a *Bcl*I/*Eco*RI subclone of the cDNA clone p5B3 coding for desmocollin DGII (Parker et al., 1991). Clone pJA70T12 is a *Bam*HI/*Eco*RI subclone of the cDNA clone pIK1 coding for desmocollin DGIV (I.A. King, unpublished data).

In-situ hybridisation

All methods were based on those of Wilkinson and Green (1990).

Preparation of sections

Human foreskin was fixed in 4% formaldehyde in PBS overnight, dehydrated in ethanol and subsequently embedded in paraffin wax. Cut ribbons of 6 μm sections were collected on slides subbed in 2% TESPA (3-aminopropyltriethoxysilane; Sigma) in acetone, dried at 37˚C and stored at 4˚C.

Preparation of single-stranded RNA probes

The cloned DNA sequences were linearised at a restriction site within the multiple cloning region distal to the RNA polymerase initiation site. Probes were labelled with ^{35}S-UTP (>1000 μCi/mmol; Amersham) according to standard protocols using either bacteriophage T7, T3 or SP6 RNA polymerase. The probes were treated with RNase-free DNase I, precipitated with ethanol and dissolved in 100 mM DTT at an activity of 1×10^6 to 2×10^6 cts/min per μl. The probes were diluted with 9 volumes of hybridisation buffer (50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 8, 5 mM EDTA, 10% dextran sulphate, 1 × Denhardt's solution, 0.5 mg/ml yeast tRNA) and stored until use at −70˚C.

Pre-treatment of sections

The sections were dewaxed in xylene (2 × 10 min), hydrated through a series of ethanol/water mixtures, subsequently incubated in saline (5 min) and PBS (5 min), fixed in fresh 4% paraformaldehyde in PBS and washed in PBS (2 × 5 min). The sections were overlaid with 20 μg/ml proteinase K in PBT (PBS; 0.1% Tween 20) and incubated for 5-10 min at room temperature. The sections were washed in PBS (5 min), re-fixed in 4% paraformaldehyde in PBS and dehydrated through a series of ethanol dilutions. The air-dried sections were used for hybridisation.

Hybridisation, washing of sections and autoradiography

A 25 μl sample of the hybridisation mix was applied to pre-treated sections, which were mounted with coverslips and incubated in a humid chamber at 60˚C overnight. Subsequently slides were washed in: 5 × SSC/10 mM DTT (50˚C, 60 min), 50% formamide/2 × SSC/20 mM DTT (65˚C, 30 min), NTE-buffer (0.5 M NaCl, 10 mM Tris-HCl, pH 8, 5 mM EDTA; 37˚C, 30 min), NTE-buffer containing 20 μg/ml ribonuclease A (37˚C, 30 min), NTE-buffer (37˚C, 15 min), 50% formamide/2 × SSC/20 mM DTT (65˚C, 30 min), 2 × SSC (room temperature, 15 min), 0.1 × SSC (room temperature, 15 min), and finally dehydrated through 30%, 60%, 80% and 95% ethanol, all including 0.3 M sodium acetate, followed by 100% ethanol. For autoradiography the air-dried slides were dipped in nuclear research emulsion (Ilford Ltd., Essex, UK), exposed for 2-5 days and finally processed with D19 developer (Eastman Kodak Co., Rochester, New York) (2 min), 1% glycerol/1% acetic acid (1 min) and 30% sodium thiosulphate (2 min). The slides were rinsed in distilled water, dehydrated in ethanol (70%, 100%), air-dried, stained with 0.02% toluidine blue and mounted. Photographs of the sections were taken using an Olympus Vanox-T microscope and ×20 objective with bright-field or dark-field illumination.

Controls with sense oligonucleotides showed weak, uniform hybridisation throughout the epidermis and dermis.

Preparation of anti-fusion protein antibodies

The raising of antibodies to DGI as TrpE fusion proteins has already been described (Wheeler et al., 1991b). These antibodies recognise the desmoglein-specific cytoplasmic repeat motif and cross-react with HDGC and possibly with other desmogleins (A.I. Magee, unpublished data). To raise antibodies to DGII/III, a 2084 bp fragment of the extracellular region of DGII/III was subcloned in-frame into the *Bam*HI/*Hin*dIII site of the pATH2 expression vector (Dieckmann and Tzagoloff, 1985). The TrpE fusion protein was expressed, separated in a 10% polyacrylamide gel stained with Coomassie Blue, excised and electroeluted. Rabbits were immunized as described by Wheeler et al. (1991b) and the resulting sera were preabsorbed with Sepharose-bound proteins from *Escherichia coli* expressing the pATH2 vector alone. The resulting antisera recognise both alternatively spliced forms DGII and DGIII but not DGIV/V (I.A. King, unpublished data).

To raise anti-desmoplakin antibodies as TrpE fusion proteins, a 912 bp fragment of desmoplakin was cloned into the pATH2 vector. This was accomplished by reverse transcription of 20 μg total RNA of SVK14 cells using an oligo(dT) primer according to the Amersham cDNA synthesis kit protocol, and amplification of 4 μl of this 50 μl cDNA reaction (corresponding to bp 4247-5228 of desmoplakin; Green et al., 1990) by PCR using primers JA98B (5′-TGGTAGATAGAAAGACAGGC-3′) and JA82 (5′-GTGCCCAATAGAACTACTGC-3′) under standard conditions. A 2 μl sample of the reaction mix was amplified in a second reaction using the inner primers JA95B (5′-GTAGGAAGAATTCC-CTGTGGC-3′) and JA99 (5′-ACAAGGGCCTTGTTGACAGG-3′). The 982 bp product was digested with *Bcl*I and *Hin*dIII and subsequently ligated into *Bam*HI/*Hin*dIII-digested pATH2 vector. The desmoplakin origin of the clone was confirmed by sequencing the ends. The fusion protein was expressed and processed as described above. Antibody specificity was tested by indirect immunofluorescence microscopy, Western blotting and radioimmunoprecipitation of cultured cells as described by Penn et al., (1987) and Wheeler et al., (1991b).

Other antibodies

The anti-plakoglobin antibody was mouse monoclonal PG 5.1 (Progen, Heidelberg, Germany). Monoclonal antibodies against

human DGIV/V were generated using the 46/48 kDa fragments in plantar callus (King et al., 1991). These do not cross-react with DGII/III in immunoblots of various human epidermal tissues (I.A. King, unpublished observations).

Immunostaining

Preparation of sections

Frozen sections of human foreskin, mounted in Tissue-Tek OCT compound (Miles Laboratories Inc., Napery, Illinois), were cut at 6 μm and stored at −70˚C before use.

Immunofluorescence

Sections were fixed for 30 min at room temperature in 4% paraformaldehyde in phosphate-buffered saline (PBS), followed by three washes in 0.1 M glycine and a 45 min block in PBS containing 0.1% BSA (Sigma) and 0.02% Triton X-100 (Sigma). The blocking solution was subsequently used for antibody dilution and washes. Sections were incubated with appropriate dilutions of primary antisera for 1 h at room temperature, washed for 30 min and then stained with fluorescein-conjugated species-specific secondary antisera for 1 h at room temperature. The sections were washed and mounted in 90% glycerol in PBS, pH 8.6 with the addition of 2.5% (w/v) 1,4-diazabicyclo-[2.2.2]octane (Sigma), and viewed with a Leitz Ortholux microscope using a ×50 oil-immersion lens.

RESULTS

Characterisation of antisera

Polyclonal rabbit antisera against desmoglein DGI, desmocollin DGII/III and desmoplakins were tested for reaction with a series of desmosome-expressing cell lines. Immunofluorescence localisation with all three sera showed punctate intercellular staining as expected of desmosomes (see Wheeler et al., 1991b; data not shown). Immunoprecipitation of [^{35}S]methionine-labelled cell lysates was also performed (Fig. 1). Anti-DGI sera immunoprecipitated a single major band of approx. 150 kDa from several cell lines including MDCK, SVK14 and HaCaT (lane 1). Anti-DGII/III sera immunoprecipitated a doublet corresponding to desmocollins DGII and DGIII (lane 2). This serum does not recognise desmocollin DGIV/V (I.A. King, unpublished data). Anti-desmoplakin antisera recognised both DPI and DPII (lane 4). Immunoblotting data confirmed the radioimmunoprecipitation results (data not shown).

Expression of the desmosomal plaque proteins, desmoplakin (DPI and DPII) and plakoglobin (DPIII)

The desmoplakins DPI and DPII and plakoglobin are major proteins of the desmosomal plaque. Plakoglobin (Franke et al., 1989) is related to β-catenin, a component of adherens junctions (McCrea et al., 1991; Butz et al., 1992; Peifer et al., 1992). *In situ* hybridization was performed with ^{35}S-labelled single-stranded RNA probes as described in Materials and Methods. The results (Fig. 2b and d) show that both the desmoplakin and plakoglobin genes are expressed throughout the living layers of the epidermis although expression in the basal layers is weak. There appears to be more expression suprabasally, which is to be expected since there have been reported to be 4 times more desmosomes in the granular layer of the skin than in the basal layer (Klein-Szanto, 1977). Immunofluorescence staining (Fig. 5a and b, below) shows a pattern of protein distribution similar to the pattern of gene expression, although here staining is evident in the basal layer.

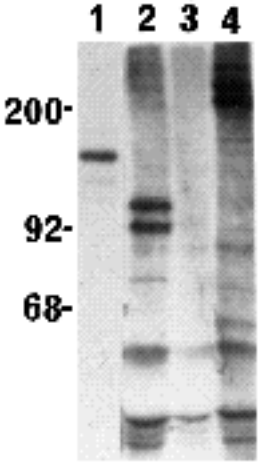

Fig. 1. Immunoprecipitation analysis of antisera. MDCK cells (lane 1) or TR131 cells (lanes 2-4) were labelled overnight with [^{35}S]methionine. Lysates were prepared in SDS, diluted and immunoprecipitated with rabbit antisera against desmoglein DGI (lane 1), desmocollin DGII/III (lane 2), desmoplakins (lane 4) or normal rabbit serum (lane 3). Molecular mass markers are myosin (200 kDa), phosphorylase (92 kDa) and bovine serum albumin (68 kDa).

Expression of keratin K14 and the bullous pemphigoid antigen I (BPAG1)

In contrast, the expression of the intermediate filament keratin K14 and the bullous pemphigoid antigen I, a component of hemidesmosomes, which link the epidermis to the underlying dermis, is only evident in the basal epidermal layer (Fig. 2f and h), as expected from previously published work (Moll et al., 1982; Mutasim et al., 1985; Regnier et al., 1985; Westgate et al., 1985; Tyner and Fuchs, 1986).

Expression of the desmocollins

Although the amino acid sequences of the two isoforms of desmocollin are closely related (68% similarity), nevertheless the DNA sequences are sufficiently different (only 60% identity) to allow specific hybridization of nucleic acid probes to one isoform only. The desmocollins DGII and DGIII are alternative splice products of a single gene (Parker et al., 1991), mapping on chromosome 9 (Arnemann et al., 1991), which differ by the insertion of a 46 bp exon with an in-frame stop codon in DGIII. DGIV and DGV are homologous alternatively spliced proteins, encoded by a single gene, mapping on chromosome 18 (J. Arnemann, unpublished data), with a similar 46 bp exon present only in DGV cDNAs. The clones used to synthesise the single-stranded RNA probes correspond to either DGII/III or DGIV/V and do not distinguish between alternatively spliced forms. Strong hybridization signals with the DGIV/V probe are observed only in the upper spinous/granular layers of the epidermis (Fig. 3b). The basal and lower spinous layers were negative. This was a similar distribution to that observed by immunofluorescence with a DGIV/V monoclonal antibody (Fig. 5d). In contrast, hybridisation signals with the DGII/III probe are less strong, with expression in the basal layer and in the cells immedi-

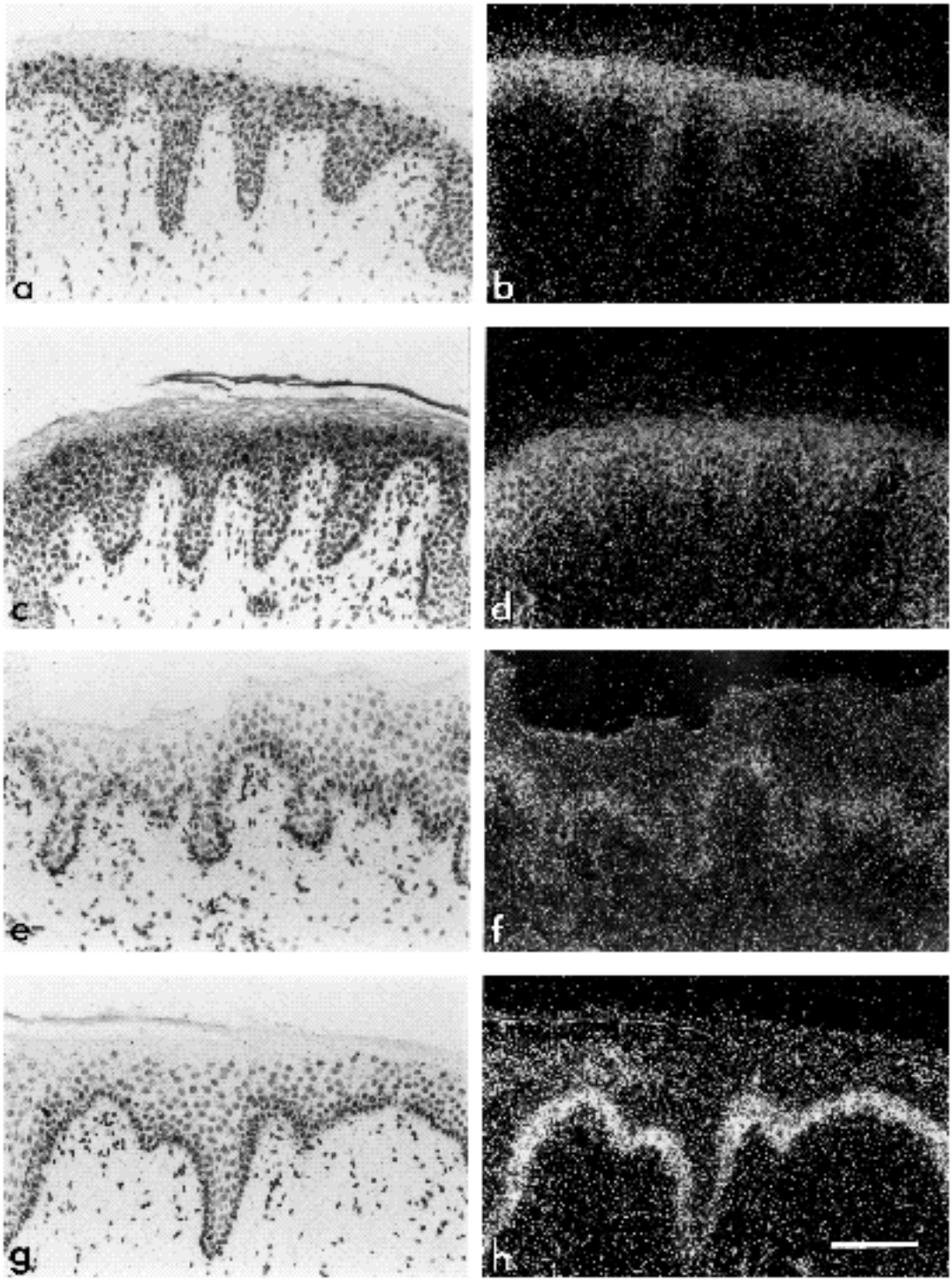

Fig. 2. *In situ* hybridisation of human foreskin, bright-field/dark-field, using [^{35}S]labelled RNA probes prepared as described in Materials and Methods. (a, b) Desmoplakin, DPI/II; (c, d) plakoglobin, DPIII; (e, f) bullous pemphigoid antigen, BPAGI; (g, h) cytokeratin K14. Bar, 100 μm.

ately suprabasal (Fig. 3d). The immunofluorescence staining obtained with DGII/III antibodies was considerably weaker than with DGIV/V reagents (Fig. 5e). Staining was generally strongest in the basal layer, becoming attenuated towards the outer layers of the tissue.

Expression of the desmogleins

The three desmogleins DGI, HDGC and PVA are, like the desmocollins, sufficiently dissimilar in their nucleic acid sequences not to cross-hybridise (% nucleic acid identity between them is not more than 59%). *In situ* hybridisation experiments (Fig. 4b) demonstrate that, amongst the

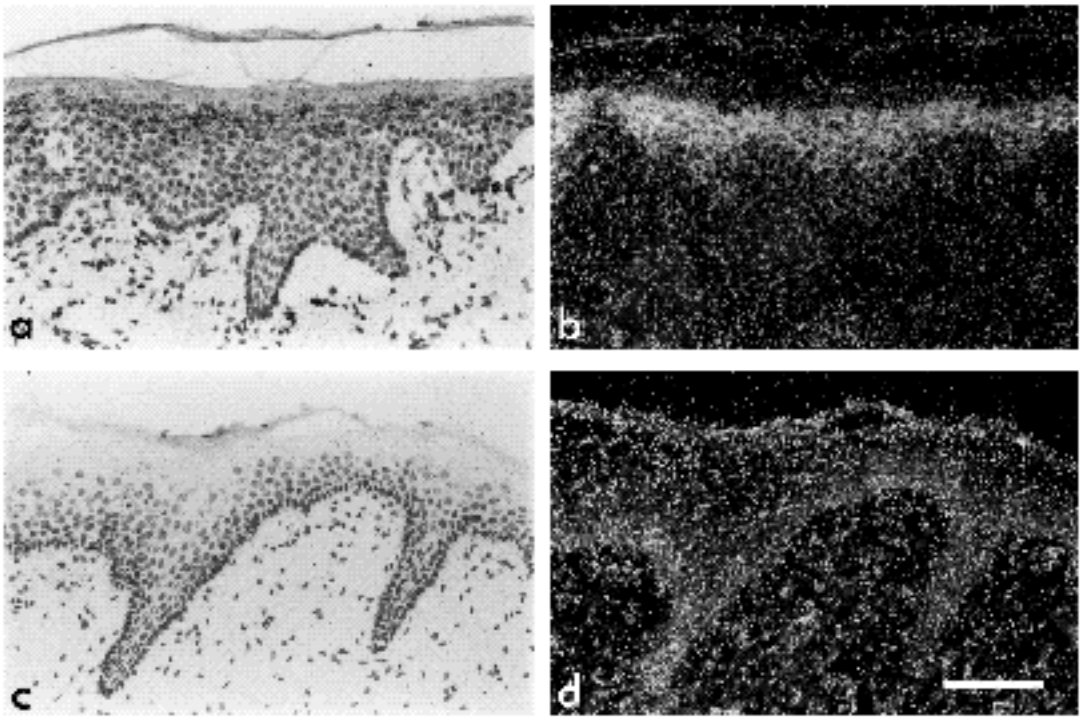

Fig. 3. *In situ* hybridisation as in Fig. 2, with RNA probes for the desmocollins: (a, b) DGIV/V; (c, d) DGII/III.

desmogleins, DGI expression is similar to that of desmoplakin and plakoglobin. (There is some staining in the stratum corneum, which was also observed with other probes, and which we attribute to non-specific binding.) DGI is the most strongly expressed of the desmogleins with weak or non-existent expression in the basal and peribasal layers. In contrast, PVA expression is more prominent in the lower spinous layers of the epidermis (Fig. 4d). Expression of HDGC, on the other hand, is difficult to ascertain from our experiments, possibly because it is a minor desmosomal component. Although HDGC appears to be expressed in basal cells it was not detected in the higher layers of the epidermis (Fig. 4f). Immunofluorescence staining with an anti-DGI antibody (Fig. 5c) reveals strong staining throughout the epidermis with weak staining of the basal cells. Compared to the *in situ* hybridisation results (Fig. 4b), staining with this antibody was stronger in the lower spinous layers probably reflecting cross-reactivity with other desmogleins such as PVA.

DISCUSSION

Epidermal differentiation involves changes in the size, shape and adhesive properties of keratinocytes and this is accompanied by well-documented changes in cytokeratin expression. Desmosomes, which maintain the structural integrity of the epidermis by linking the cytokeratin networks of neighbouring cells, are also known to undergo morphological changes during keratinisation. It now appears that these changes in desmosome form and adhesive properties parallel molecular changes in the expression of particular desmosome components concerned with epidermal cell adhesion.

From the results of cDNA cloning and sequencing it is now known that there are a number of different isoforms of the putative adhesion molecules of the desmosome, the desmocollins and the desmogleins. The relative adhesive capacities of these different isoforms have yet to be ascertained. In the present paper we have shown that the expression of these isoforms differs. In particular, the major transcripts are DGIV/V and DGI. DGIV/V expression is restricted to the upper spinous/granular layers, whilst DGI is expressed suprabasally in a similar distribution to the plaque proteins desmoplakin and plakoglobin. In contrast, desmocollin DGII/III and possibly the desmoglein HDGC are expressed in the basal and peribasal layers; in the case of DGII/III, this is also evident from the immunofluorescence staining with anti-fusion protein antibodies, which are isoform-specific. The low level of expression of DGI, desmoplakin and plakoglobin in the basal layers may be a reflection of the low numbers of desmosomes there, or there may be a solely suprabasal distribution of these transcripts. The anti-DGI antibody shows more intense staining in the lower spinous layers, consistent with a possible cross-reaction with PVA. It will be most interesting to determine the differences in expression between DGII and DGIII, and between DGIV and DGV, and also to know what differences there are in the function of these different isoforms.

Besides DGI, the desmoglein PVA (pemphigus vulgaris antigen) is also expressed suprabasally with little or no expression in the basal layers. Although not as prevalent as DGI, it was expressed more strongly in the lower spin-

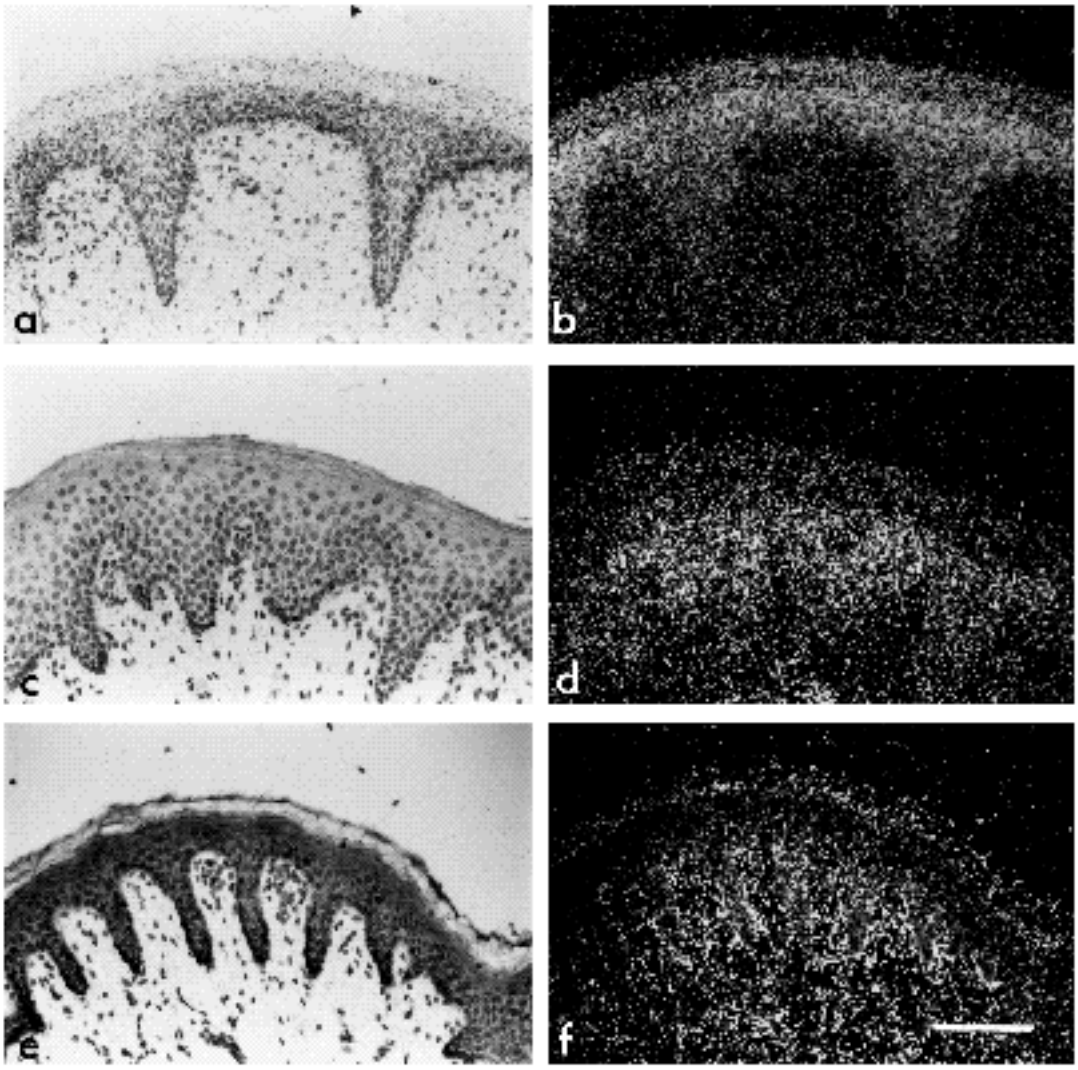

Fig. 4. *In situ* hybridization as in Fig. 2, with RNA probes for the desmogleins: (a, b) DGI; (c, d) PVA; (e, f) HDGC.

ous layers than the major desmoglein. DGI is probably the autoantigen in the blistering skin disease pemphigus foliaceous (Koulu et al., 1984; Stanley et al., 1986), in which the split in the epidermis is more superficial than in pemphigus vulgaris (Korman, 1990). Some inherited bullous skin diseases, as opposed to autoimmune disorders, such as the autosomal dominant Darier's disease and Hailey-Hailey disease, may also exhibit defects of desmosomal cadherin structure or expression (Burge, 1989; Burge et al., 1990, 1991), although no changes have yet been found by immunofluorescence staining (Burge and Garrod, 1991). It may of course be that the antibodies used cross-reacted with more than one isoform, so that differences in one desmosomal cadherin were obscured. *In situ* hybridisation now provides a further way of testing for this. None of the autoimmune skin diseases has been shown to involve the desmocollins; one explanation, given that both desmogleins and desmocollins are likely to be adhesive molecules, is that the desmocollins are of more fundamental importance and defects in them may have disastrous consequences.

There does not appear to be any particular change in the expression pattern of the genes coding for the two plaque proteins, desmoplakin and plakoglobin, other than that there is more expression suprabasally, paralleling the increased abundance of desmosomes in the spinous layer compared with the basal layers (Klein-Szanto, 1977). It was, however, difficult to demonstrate expression in the basal layer; as mentioned above for DGI and PVA, whether this is real or not is difficult at present to decide.

Changes in desmosomal composition appear therefore to be restricted to the desmosomal glycoproteins, although certain plaque proteins have been reported to be present only in stratified tissue (see Introduction). In the basal layers of the epidermis where desmosomes are relatively sparse HDGC and DGII/III appear to be the predominant desmosomal cadherin species. The fate of desmoglein HDGC in the upper layers is not yet clear. However, desmocollin DGII/III probably persists in the desmosomes of spinous cells, since it could be detected in these layers by fluorescence. It is not clear whether it persists as an intact, functionally adhesive, molecule in the upper living layers. Pro-

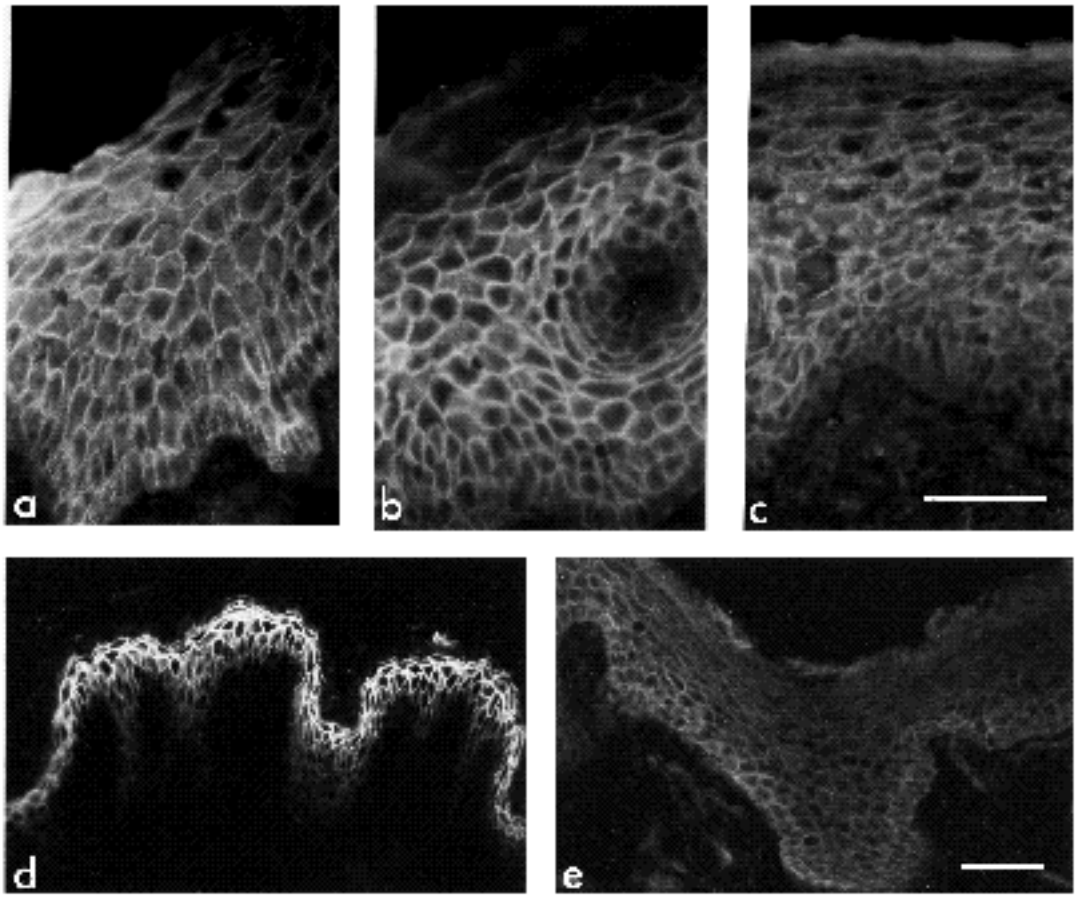

Fig. 5. Immunofluorescence staining of human foreskin with antibodies against the following proteins: (a) desmoplakin; (b) plakoglobin; (c) desmoglein DGI; (d) desmocollin DGIV/V; (e) desmocollin DGII/III. Bar, 50 μm.

teolytic fragments of DGII/III have been detected in the terminally differentiated layers of human epidermis (I.A. King, unpublished data). In the lower spinous layers there appears to be strong expression of PVA and increasing expression of DGI but little expression of DGIV/V. Expression of DGI continues in the upper spinous/granular layers where DGIV/V expression becomes apparent by both *in situ* hybridisation and immunofluorescence with monoclonal antibodies. The latter contrasts with the results of Parrish et al. (1986), who obtained polyclonal desmocollin antibodies that stained all suprabasal layers of the epidermis including lower spinous layers. This discrepancy may reflect cross-reactivity of those polyclonal antibodies with another, unidentified, desmocollin isoform, possibly expressed, like PVA, mainly in the lower spinous layers. Transition to the stratum corneum is associated with loss of desmosome-mediated cell adhesion and concomitant degradation of desmosomal cadherins (King et al., 1987, 1989). It does not appear at this point therefore, that the changes in desmosomal cadherin expression during stratification exactly mirror changes in cytokeratin expression and, if anything, the changes in expression of the desmosomal cadherins are not as clear cut as the changes in cytokeratin expression.

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