

# The Bovine Desmocollin Family: A New Gene and Expression Patterns Reflecting Epithelial Cell Proliferation and Differentiation

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**Abstract.** We have discovered a third bovine desmocollin gene, DSC3, and studied expression of all three desmocollin genes, DSC1, 2, and 3, by Northern blotting, RT-PCR and *in situ* hybridization. DSC1 is strongly expressed in epidermis and tongue papillae, showing a "skin"-type pattern resembling that previously described for keratins 1 and 10. Expression is absent from the epidermal basal layer but appears in the immediate suprabasal layers and continues uniformly to the lower granular layer. In tongue epithelium, expression is suprabasal and strictly localized to papillae, being absent from interpapillary regions. In other epithelia low level DSC1 expression is detectable only by RT-PCR. The distribution of Dscl glycoproteins, detected by an isoform-specific monoclonal antibody, closely reflects mRNA distribution in epidermis and tongue. DSC2 is ubiquitously expressed in epi-

thelia and cardiac muscle. In stratified epithelia, expression appears immediately suprabasal, continuing weakly to the lower granular layer in epidermis and to just above half epithelial thickness in interpapillary tongue, oesophageal, and ruminal epithelia. DSC3 expression is restricted to the basal and immediately suprabasal layers in stratified epithelia. In deep rete ridges DSC expression strikingly resembles the distribution of stem, transit-amplifying, and terminally differentiating cells described by others. DSC3 expression is strongly basal, DSC2 is strong in 5–10 suprabasal layers, and then weakens to be superseded by strong DSC1. These results suggest that desmocollin isoform expression has important functional consequences in epithelial proliferation, stratification, and differentiation. The data also provide a standard for nomenclature of the desmocollins.

DESMOSOMES are punctate adhesive intercellular junctions associated with the intermediate filament network in epithelia, cardiac muscle, meninges, and follicular dendritic cells (for review see Garrod et al., 1990; Schwartz et al., 1990; Legan et al., 1992; Garrod and Collins, 1992; Garrod, 1993). The desmosomal glycoproteins, desmocollins and desmoglein, form two sub-families of the cadherin family of calcium dependent cell-cell adhesion molecules (for review see Magee and Buxton, 1992; Buxton and Magee, 1992). A new nomenclature for desmosomal cadherins has now been proposed (Buxton et al., 1993).

The adhesive role of the desmocollins (from the Greek  $\delta\epsilon\sigma\mu\sigma$  = link, and  $\kappa\lambda\lambda\alpha$  = glue) was first suggested by

inhibition of desmosome assembly in Madin Darby Bovine Kidney (MDBK)<sup>1</sup> cells with Fab' fragments of a polyclonal antiserum (Cowin et al., 1984). Their relatedness to cadherins was demonstrated by NH<sub>2</sub>-terminal protein sequencing (Holton et al., 1990). Subsequent sequencing of bovine and human cDNA clones showed that they resemble the structure of cadherins in their extracellular regions (Collins et al., 1991; Koch et al., 1991, 1992; Mechanic et al., 1991; Parker et al., 1991). The cytoplasmic domains are unique among cadherins, showing size heterogeneity (Parrish et al., 1990) due to alternative mRNA splicing (Collins et al., 1991; Parker et al., 1991). The larger splice variant, the "a" form, is longer than the 'b' form in the cytoplasmic domain. The COOH-terminal region of the a form shows similarity with the cadherins and also with part of the desmoglein cytoplasmic domain (Collins et al., 1991; Parker et al., 1991). The cytoplasmic domains of both desmocollins and desmoglein are presumably specialized for participation in the structure of the desmosomal plaque. In support of this theory, expres-

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1. Abbreviations used in this paper: AP, alkaline phosphatase; IPTG, isopropyl- $\beta$ -D-thiogalactopyranoside; MDBK, Madin Darby Bovine Kidney; SC, stem cells; TA, transit amplifying; TD, terminally differentiated.

sion of connexin-desmocollin chimeras in A431 cells demonstrates that the cytoplasmic region of the a form supports plaque assembly (Troyanovsky et al., 1993).

Two different bovine desmocollins arising from distinct genes have been described (Collins et al., 1991; Koch et al., 1991, 1992; Mechanic et al., 1991). These are referred to as Dsc1 and Dsc2, and the corresponding genes as DSC1 and DSC2 (Buxton et al., 1993). The first published human desmocollin sequence (Parker et al., 1991) was called DGII/III, II, and III denoting the alternatively spliced forms. It shows 76% protein sequence identity with bovine Dsc2 but it is not clear if these are interspecies homologues. Sequence data from epidermal peptide fragments showed the existence of a second human desmocollin isoform (previously called DGIV/V) apparently homologous to bovine Dsc1 (King et al., 1991). This was subsequently confirmed by cDNA sequencing (Theis et al., 1993; King et al., 1993a).

Here we describe a third bovine desmocollin isoform, Dsc3, and show it is the product of a distinct gene, DSC3. We show that its cytoplasmic domain shows size heterogeneity due to alternative mRNA splicing typical of other desmocollins (Parrish et al., 1990; Collins et al., 1991; Parker et al., 1991; Koch et al., 1992). In addition, we present a detailed study of the expression of the bovine desmocollin genes by Northern blotting, RT-PCR, in situ hybridization, and monoclonal antibody labeling. This shows that they have distinct patterns of tissue expression and of differentiation-related expression in stratified epithelia. A particularly striking result is that expression of the novel gene, DSC3, appears to be restricted to stratified epithelia where it is strongly associated with the basal layers.

## Materials and Methods

### Isolation of Total RNA

Bovine tissues were dissected and frozen in liquid nitrogen at the abattoir. Frozen tissue (2 g) was pulverized in a mortar and pestle cooled with liquid nitrogen and total RNA isolated from the ground tissue (Chomczynski and Sacchi, 1987). Total RNA was similarly isolated from confluent monolayers of MDBK cells.

### Reverse Transcriptase-mediated Polymerase Chain Reaction

The following PCR primers were used (internal restriction sites underlined); KS2, GGGAATTC(C/T)TNGGNA(A/G)TGGGCNAT (EcoRI), 256-fold redundant, 1,280 pmol/reaction; KA3, GGGAATTCGTNC(G/T)-(A/G)AA(T/C)TTNGG(T/C)TC (EcoRI), 256-fold redundant, 1,280 pmol/reaction; A2, GCGAATTCAGTGTGTCCTCTAAC (EcoRI), 100 pmol/reaction; YSI, GCGGATCCCAGTCAACACGCTAAC (BamHI), 100 pmol/reaction; YA2, GCGGATCCA(A/G)NCCNTC(T/C)TC(T/C)TC(T/C)TG (BamHI), 256-fold redundant, 1,280 pmol/reaction.

Random-primed first strand cDNA synthesis (Gubler and Hoffmann, 1983) was carried out on total RNA (1–2 µg), using cDNA synthesis kits (Boehringer Mannheim Corp., Amersham International). An aliquot (1 µl) of the first strand cDNA reaction was added directly to the PCR mix containing 0.2 mM each dNTP, 2.5U Taq DNA polymerase (Promega Corp., Madison, WI), sense and anti-sense primers in 100 µl 1× Taq DNA polymerase buffer (50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 100 mM Tris-HCl (pH 9.0), 1% Triton X-100). A Techne PHC-3 Thermal Cycler was used with the following cycling times and temperatures; KS2 and A2, 94°C for 2 min, followed by five cycles of 50°C for 1 min, 72°C for 1 min, 94°C for 45 s, and 30 cycles of 60°C for 1 min, 72°C for 1 min, 94°C for 45 s, and then 72°C for 5 min; YSI and YA2, as KS2 and A2 except that after the first five cycles annealing was at 65°C. Reaction products were separated by 2.0% agarose gel electrophoresis and visualized by staining with ethidium bromide (0.5 µg/ml).

### DNA Sequencing and Sequence Analysis

PCR products were subcloned into pBluescript II SK+ (Stratagene, La Jolla, CA) using internal restriction sites incorporated into the primers. Plasmid DNA was prepared from at least three separate transformants per PCR product using Magic Miniprep columns (Promega Corp.). Plasmid DNA was denatured (Sambrook et al., 1989) and sequenced (Sanger et al., 1977) in both directions using T3 and T7 primers, and sequenase V2.0 (USB, Cleveland, OH). Sequence analysis was carried out using PC-Gene (Intelligenetics Inc., Mountain View, CA).

### Southern Blotting

Southern blots of MDBK cell genomic DNA digested with EcoRI, KpnI, BglII, HincII, PstI, BamHI, EcoRV, or SstI were prepared and probed as described in Collins et al. (1991). Between hybridizations, probe was stripped off the filter by washing in 0.4 M NaOH for 45 min at 45°C followed by 0.1× SSC, 0.1% SDS, 0.2 M Tris-HCl (pH 7.5) for 30 min at 45°C.

### Northern Blotting

Aliquots (30 µg) of total RNA from bovine nasal epidermis, tongue, oesophagus, trachea, lung, rumen, intestine, liver, kidney, gall bladder, uterus, cultured MDBK cells, cardiac muscle, and skeletal muscle were separated by formaldehyde gel electrophoresis (Sambrook et al., 1989), transferred to Hybond-N (Amersham International) and hybridized to <sup>32</sup>P-labeled probes at 47°C in 50% formamide (Angst et al., 1990). Blots were washed twice for 15 min each in 2× SSC at 65°C, for 30 min in 2× SSC, 0.1% SDS at 65°C, and for 10 min in 0.1× SSC at 65°C and autoradiographed. Probe was stripped off the filter between hybridizations by washing for 1–2 h in 5 mM Tris-HCl (pH 8.0), 2 mM EDTA, 0.1× Denhardt's solution at 65°C.

### Probe Preparation and Labeling

For blotting, inserts from pBluescript subclones of the KS2-A2 primed Dsc2b and Dsc3b PCR products, and the 688-bp BglII-PstI restriction fragment of our Dsc1b cDNA clone (Collins et al., 1991) were purified from gels using GeneClean or Mermaid (Bio101). Inserts were then labeled (Feinberg and Vogelstein, 1983) using random primers and  $\alpha^{32}$ P-dCTP (Multi-Prime, Amersham International) and unincorporated label removed on Nensorb columns (Dupont, Wilmington, DE).

For in situ hybridization, KS2-A2 primed Dsc2b and Dsc3b PCR products, and the 688-bp BglII-PstI restriction fragment of our Dsc1b cDNA clone (Collins et al., 1991) were subcloned into pBluescriptII SK+ and <sup>35</sup>S-labeled sense and anti-sense RNA transcribed in vitro with T7 and T3 RNA polymerases from a commercial kit (Stratagene). Transcripts were prepared for use as in situ hybridization probes according to Wilkinson and Green (1990).

### In Situ Hybridization

Freshly collected bovine tissue samples were fixed in 4% (wt/vol) paraformaldehyde in PBS overnight at 4°C, dehydrated, and embedded in paraffin wax according to Sasoon et al. (1988). Serial sections (6 µm) were spread on 3-aminopropyltriethoxysilane-treated slides (Wilkinson and Green, 1990) and dried overnight at 37°C. In situ hybridization was carried out according to Wilkinson and Green (1990).

### Construction of Expression Plasmids and Detection of Fusion Proteins

Construction of plasmid pUC18-CN35-XbaI was described by Dmochowski et al. (1993). This plasmid encodes a protein comprised of the first 6 amino acids of  $\beta$ -galactosidase fused in-frame to 768 amino acids of bovine Dsc1b, including 61 amino acids of the propeptide and the entire mature protein sequence.

Plasmid pUC18-BMDCT2-Sac/Hind was constructed as follows: Clone BMDCT2-DC4.1 (Koch et al., 1992) was cut with SacI and briefly digested with S1 nuclease. A SacI (blunt-ended)-HindIII fragment was then cloned into pUC18 cut with HincII and HindIII. Resulting plasmids were sequenced to identify in-frame fusions. One plasmid, pUC18-BMDCT2-Sac/Hind, was chosen for further study. This plasmid encodes a protein comprised of the first 6 amino acids of  $\beta$ -galactosidase fused in-frame to 763 amino acids of bovine Dsc2b, including 43 propeptide amino acids and the entire mature protein sequence.

Plasmids pUC18-CN35-XbaI and pUC18-BMDCT2-Sac/Hind were transformed into *E. coli* XL1-blue. Transformants were grown for 3 h in the presence of 1 mM isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) to induce expression of the fusion proteins. Cells were harvested, lysed in Laemmli's sample buffer, and boiled for 3 min. Proteins were separated by SDS-PAGE (Laemmli, 1970) and transferred to nitrocellulose. Blots were blocked in 5% skimmed milk and probed with antibodies raised against bovine nasal epidermal desmocollins. The guinea pig polyclonal antiserum DG2 (diluted 1:5,000) was detected with alkaline phosphatase (AP) conjugated anti-guinea pig IgG (1:15,000). The mouse monoclonal antibodies 52-3D (1:200) and 07-4D (1:50) were detected with biotinylated anti-mouse IgG (1:15,000) followed by ABC-AP reagent (Vector Labs., Burlingame, CA). In all cases color was developed using BCIP/NBT substrate (Vector Labs.).

### Antibodies and Immunostaining

Primary antibodies used were a monoclonal, 07-4D, raised against total nasal epidermal desmocollins (Holton et al., 1991); an anti-desmocollin monoclonal, 52-3D, with wide tissue cross reactivity (Parrish et al., 1990; Collins et al., 1991; Garrod and Collins, 1992); 11-5F, an anti-desmoplakin monoclonal (Parrish et al., 1987); and a guinea pig polyclonal antiserum, DG2, raised against bovine nasal epidermal desmocollins (Mattey et al., 1990).

Immunostaining of cryosections was carried out as described in Cowin and Garrod (1983) using an FITC-conjugated goat anti-mouse IgG secondary antibody (Sigma Chem. Co., St. Louis, MO). 07-4D staining on filiform papillae was detected using a biotinylated rabbit anti-mouse IgG secondary antibody and ExtrAvidin-FITC conjugate (Sigma Chem. Co.). Sections were mounted in O.C.T. compound (Tissue Tek) and examined using a Zeiss Axioplan microscope. Sections of filiform papillae stained with 07-4D were examined using an MR600 confocal microscope (BioRad Labs., Hercules, CA).

## Results

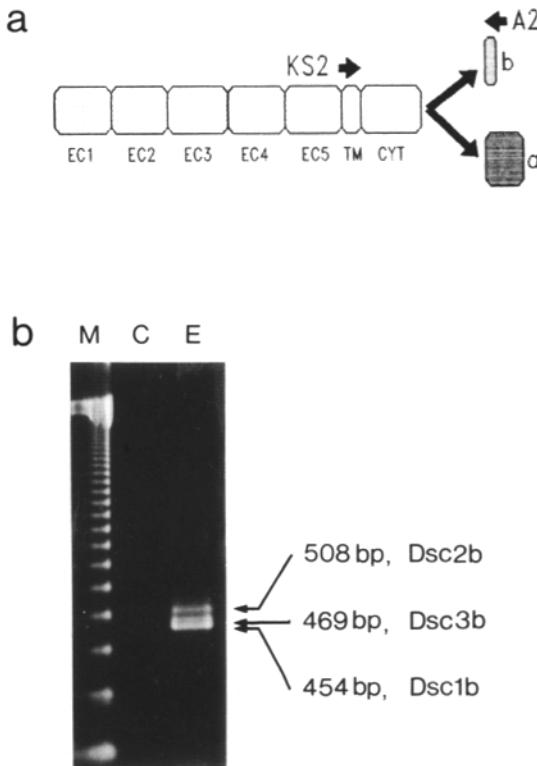
### A Third Bovine Desmocollin Gene

We amplified sequences related to Dsc1b (Collins et al., 1991) by RT-PCR using a redundant 5' primer, KS2, in the transmembrane domain of Dsc1 and a non-redundant 3' primer, A2, from the conserved 46 bp alternatively spliced exon encoding the COOH terminus of Dsc1b (Fig. 1 a). These primers amplify a region of the Dsc cytoplasmic domain common to both splice variants (Fig. 1 a, CYT). Three products of 508, 469, and 454 bp were generated from bovine nasal epidermis total RNA (Fig. 1 b). These products were subcloned and three independent clones sequenced for each product. The 454-bp product was identical to Dsc1b (Collins et al., 1991) and the 508-bp product to Dsc2b (Koch et al., 1992). The sequence of the 469-bp product (see below) was similar to, but distinct from, the known bovine desmocollins, having 67.8% DNA sequence identity with Dsc1b and 74.8% identity with Dsc2b. This product was designated Dsc3b.

Dsc1 and Dsc2 are encoded by single genes, DSC1 and DSC2 (Collins et al., 1991; Koch et al., 1992; Buxton et al., 1993). Southern blotting of MDBK cell genomic DNA with the Dsc3b product gave a unique pattern (Fig. 2) indicating that Dsc3 mRNA is encoded by a distinct single gene, tentatively named DSC3.

### Dsc3 Is Alternatively Spliced in the Cytoplasmic Domain

To determine whether Dsc3 is alternatively spliced in the cytoplasmic domain, sequence spanning the predicted splice site was obtained by reverse transcriptase-mediated polymerase chain reaction (RT-PCR) using primers YS1 and YA2

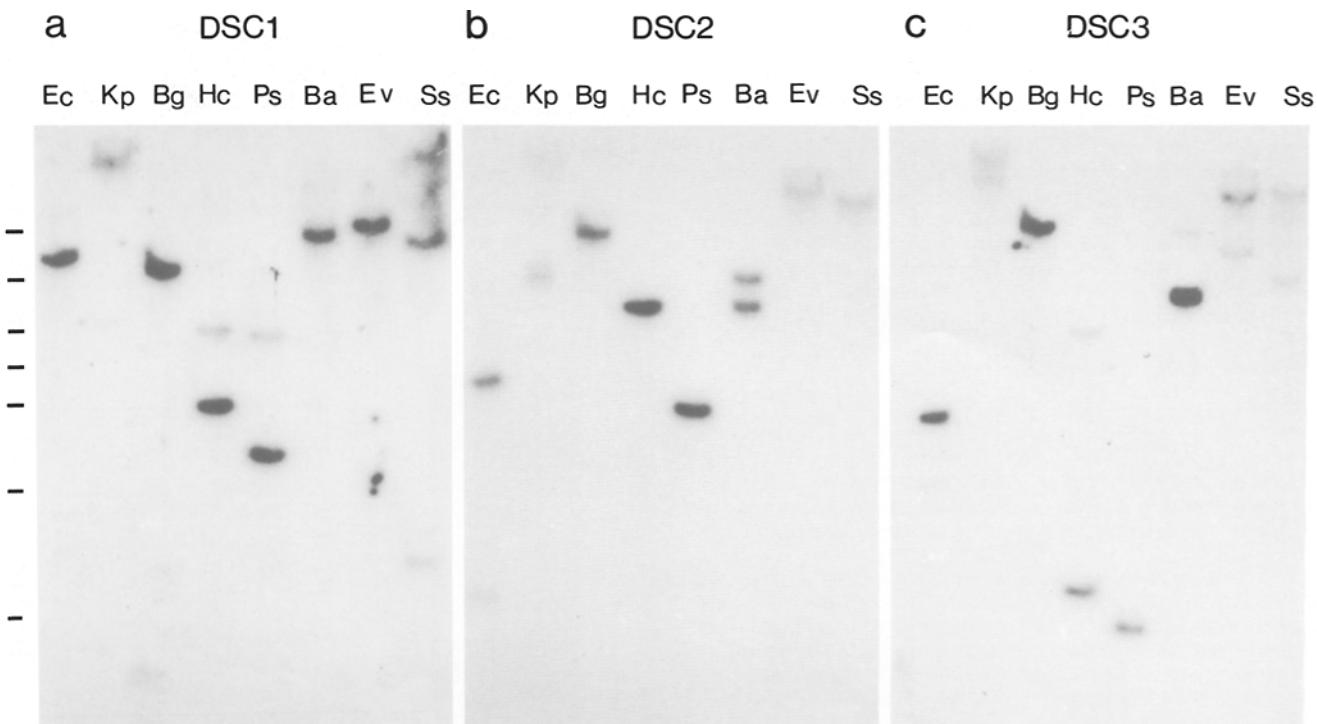


**Figure 1.** Detecting new desmocollin type by PCR. (a) Schematic structure of Dsc1. The five extracellular regions (EC1-5), the transmembrane domain (TM), and part of the cytoplasmic domain (CYT) are common to both splice variants of Dsc1. The alternative COOH-termini of the a and b splice variants complete the structure. RT-PCR was carried out using primers KS2, from the transmembrane domain, and A2, from the Dsc1b COOH terminus (arrowed). (b) PCR products separated by gel electrophoresis; lane M, markers (123 bp ladder, GIBCO-BRL); lane C, control RT-PCR reaction without RNA; lane E, RT-PCR reaction using epidermal total RNA. Specific products of 508, 469, and 454 bp are arrowed.

(Fig. 3). YS1 is a unique sense primer derived from the Dsc3b sequence. YA2 is a redundant antisense primer based on the conserved amino acid sequence QEEDGL in the COOH-terminal regions of Dsc1a and Dsc2a. Two products of 420 and 377 bp differing only in the presence or absence of a 43-bp sequence were obtained, providing evidence for alternative splicing. It should be noted that the alternatively spliced exon in both Dsc1b and Dsc2b is 46-bp long and encodes a conserved 11-amino acid COOH terminus. In contrast, the 43-bp DSC3 exon encodes a 8-amino acid COOH terminus (Fig. 3). Of these, the first seven amino acids are identical to the corresponding residues in Dsc1b and Dsc2b, but Dsc3b terminates with a glycine residue in place of the conserved motif L(V/I)KN. In the absence of the 43-bp exon, the open reading frame continues for at least 140 bp, and encodes part of the COOH-terminal region of the a splice variant (Fig. 3).

### Amino Acid Sequence Comparisons

Identities between the deduced partial amino acid sequence of Dsc3a and the corresponding regions of other known des-



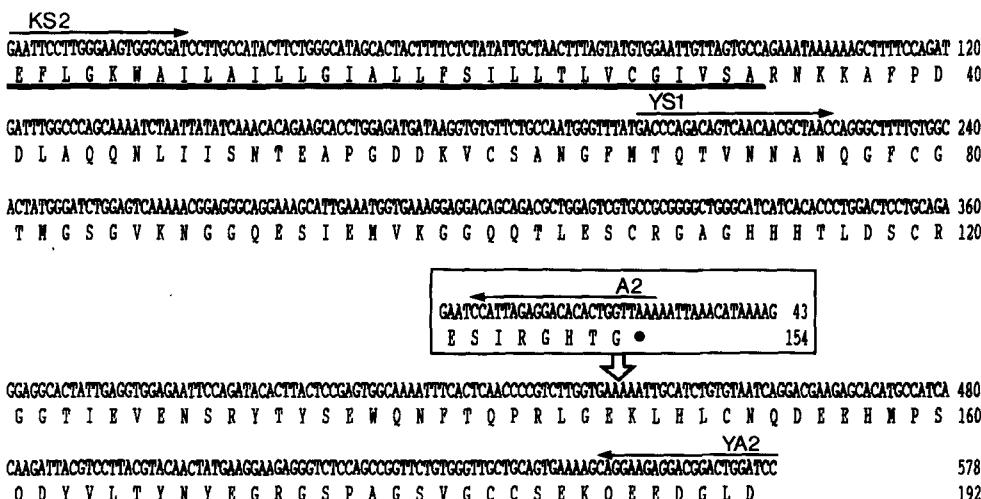
**Figure 2.** Type 1, 2, and 3 desmocollins come from different genes. A Southern blot of MDBK cell genomic DNA was sequentially hybridized with probes specific for (a) DSC1; (b) DSC2; and (c) the 469-bp Dsc3b product. Each probe gave a unique banding pattern, including single bands in many of the lanes. *Ec*, EcoRI; *Kp*, KpnI; *Bg*, BglII; *Hc*, HincII; *Ps*, PstI; *Ba*, BamHI; *Ev*, EcoRV; *Ss*, SstI. Markers: 23, 9.6, 5.8, 4.3, 3.5, 2.3, and 1.4 kb.

mocollins are shown in Table I. It is not clear from these scores whether the human DGII/III sequence is the homologue of bovine Dsc2 or Dsc3. However, multiple sequence alignment of desmocollin deduced amino acid sequences (Fig. 4) reveals that both human DGII/III and bovine Dsc3 contain two partially conserved repeats of the sequence HTLDSCRGG, whereas bovine Dsc2 contains three such

repeats (thick overlines in Fig. 4), so, in this respect, human DGII/III more closely resembles bovine Dsc3.

#### Tissue Expression of the Three Desmocollin Genes

Tissue specific expression was investigated by Northern blotting of total RNA from stratified, pseudostratified, transitional, and simple epithelial tissues, cardiac muscle, and



**Figure 3.** Sequence of type 3 desmocollin. Partial DNA and derived amino acid sequences of Dsc3a and b splice variants determined from the overlapping PCR products generated by primers KS2-A2 and YS1-YA2. The PCR primers are arrowed. KS2 and A2 primed the initial Dsc3b product, while YS1 and YA2 primed the sequence spanning the splice site. The putative 43-bp exon encoding the COOH terminus of the b splice variant of Dsc3 is boxed, and the splice site is arrowed. The filled circle marks an in-frame stop codon. The predicted transmembrane domain is underlined. These sequence data are available from EMBL/GenBank/DDBJ under accession number X75783.

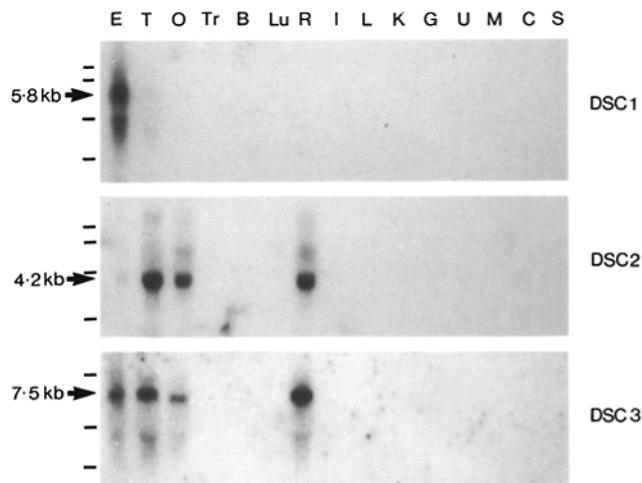
*Table I. Comparison of Derived Amino Acid Sequences of Bovine and Human Desmocollins*

BDsc1a	BDsc2a	BDsc3a	HDsc1a	HDGII
51.6	72.2	51.3	54.0	
56.0	48.7	79.7		
88.0				
56.5	79.5			

All scores are % identities over the length of the partial amino acid sequence for bovine Dsc3a (Fig. 3) and the corresponding regions of other known desmocollins. (Bovine Dsc1a, BDsc1a, Collins et al., 1991; bovine Dsc2a, BDsc2a, Koch et al., 1992; bovine Dsc3a, BDsc3a, this study; human Dsc1a, HDsc1a, Theis et al., 1993; human DGII, HDGII, Parker et al., 1991).

MDBK cells. The non-desmosome bearing tissue skeletal muscle was used as a negative control. The 5.8-kb Dsc1 mRNA was detected strongly in epidermis (Collins et al., 1991) and weakly in tongue epithelium, but was undetectable in all other epithelia, cardiac, and skeletal muscle, even after overexposure of the blot (Fig. 5). Dsc2 mRNA was detected as a broad band at 4.2 kb in tongue, consistent with Koch et al. (1992) who report the Dsc2 mRNA as a doublet of 4.2 and 4.0 kb in tongue mucosa. The Dsc2 mRNAs were also detected strongly in oesophagus and rumen (stratified), and weakly in epidermis and cardiac muscle (Fig. 5). The Dsc3 mRNA was detected strongly in stratified epithelia (epidermis, tongue, oesophagus, and rumen) but not elsewhere (Fig. 5). The Dsc3 mRNA was 7.5 kb in epidermis and tongue, but 7.2 kb in oesophagus and rumen, suggesting some difference in processing of the gene transcripts in these tissues.

Since expression of the Dsc mRNAs was only detectable in a subset of tissues by Northern blotting, tissue expression was studied further by RT-PCR using the KS2 and A2 primers. Blank isolations carried out between each preparation of total RNA were used as controls in the RT and PCR



**Figure 5.** Northern blot analysis of DSC1, 2, and 3 expression. A single Northern blot of aliquots (30 µg) of total RNA from bovine epithelial tissues and one cell line was sequentially hybridized with probes specific for Dsc1, Dsc2, and Dsc3 mRNAs. The blots are labeled with the DSC type on the right, and the size of the specific mRNA is arrowed on the left of the figure. The resolving power of the formaldehyde gels used was insufficient to separate RNAs specific for the a and b splice variants, which differ by only 43 or 46 bases. Tissues probed; *E*, epidermis; *T*, tongue; *O*, oesophagus; *Tr*, trachea; *B*, bladder; *Lu*, lung; *R*, rumen; *I*, intestine; *L*, liver; *K*, kidney; *G*, gall bladder; *U*, uterus; *M*, MDBK cells; *C*, cardiac muscle; *S*, skeletal muscle. Markers 9.5, 7.5, 4.4, and 2.4 kb (BRL RNA Ladder).

reactions. In addition no products were obtained from skeletal muscle which lacks desmosomes (Fig. 6). On ethidium bromide-stained gels bands corresponding in size to the DSC1 product were visible in epidermis, tongue, intestine,

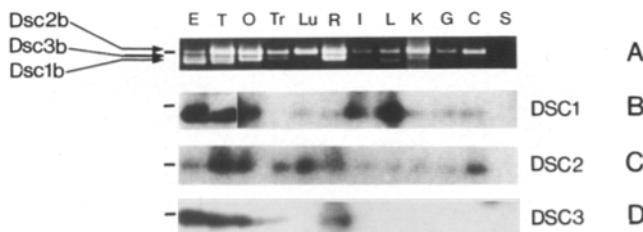
HDsc1a	VILGKWAIIALVLSVLLCILPTCPCTVAKTVK-KCPPEDI	594
BDsc1a	VPLGKWAIIALVLSVLLCILPTCPCTVAKTVK-KCPPEDV	729
BDsc2a	VILGKWAIIALVLLGIALLFCILPTLVCGATTGADNPKVPPDDL	683
BDsc3a	KFLGKWAIIALVLLGIALLFSILLALVCGIV-SA-RKKKAPPDL	42
HDsc3a	VOLGKWAIIALVLLGIALLFCILPTLVCGASCTS-KQPKVIPDDL	597

HDsc1a	AQQNLIVSNTRPGEVVEFTANIRLPMTQTSNCDCTSMSVGTVGGQGIKT---QQSPEVKGG-----	YTLDSMEGGGHQTLSEVKVGVG-QDGTGRVAYTD	684
BDsc1a	AQQNLIVSNTRPGEVVEFTANIRLPMTQTSNCDCTSMSVGTVGGQGIKT---QQSPEVKGG-----	YTLDSMEGGGHQTLSEVKVGVG-DTGRVTTSD	817
BDsc2a	AQQNLIVSNTRPGEVVEFTANIRLPMTQTSNCDCTSMSVGTVGGQGIKT---QQSPEVKGG-----	YTLDSMEGGGHQTLSEVKVGVG-DTGRVTTSD	789
BDsc3a	AQQNLIVSNTRPGEVVEFTANIRLPMTQTSNCDCTSMSVGTVGGQGIKT---QQSPEVKGG-----	YTLDSMEGGGHQTLSEVKVGVG-DTGRVTTSD	135
HDsc3a	AQQNLIVSNTRPGEVVEFTANIRLPMTQTSNCDCTSMSVGTVGGQGIKT---QQSPEVKGG-----	YTLDSMEGGGHQTLSEVKVGVG-DTGRVTTSD	690

HDsc1b	ESIRGHTLILKN	706
BDsc1b	ESIRGHTLVKN	839
BDsc2b	ESIRGHTLVKN	809
BDsc3b	ESIRGHTG	154
HDsc3b	ESIRGHTLILKN	712
*****		

HDsc1a	WQSTPQRIGE KVYLQCGQKEHKKCDEYVFSYNEBCKGSVAGCSDRQKEEGLPLDNLLEPKFRTLAETCIIK•	748
BDsc1a	WNHTPQRIGE KVYLQCGQKEHKKCDEYVFSYNEBCKGSVAGCSDRQKEEGLPLDNLLEPKFRTLAETCVKK•	893
BDsc2a	WTTTPQRIGE KVYQCDQDNTEHQDTVLTNYEGRSAAAGSVGCSCSERQEEGLEFLDNLQGPKPAATLAECCR•	875
BDsc3a	WNQTPQRIGE KLRICQDKEHMPQDVVLTNYEGRSSPAGSVGCSEKQKEQGID-----	192
HDsc3a	WQSTPQRIGE KVYLQCGQKEHKKCDEYVFSYNEBCKGSVAGCSDRQKEEGLPLDNLLEPKFRTLAETCIIK•	772

**Figure 4.** Multiple alignment of the derived amino acid sequences of bovine and human desmocollins. Sequences are labeled as in Table I. An alignment of the Dsc b COOH termini is boxed and the splice site arrowed. Sequences are numbered according to the original publications. Predicted transmembrane regions are underlined. Partially conserved repeats of the sequence HTL-DSCRGG are overlined. The COOH-terminal end of the proteins are marked with a filled circle. Residues identical in all five sequences are marked with (\*). Conservative amino acid substitutions are marked with (.).



**Figure 6.** Tissue specificity of DSC1, 2, and 3 expression determined by RT-PCR analysis. RT-PCR using primers KS2 and A2 was carried out on 1–4  $\mu$ l of random primed first strand cDNA synthesized from total RNA (1  $\mu$ g) from each tissue. (A) PCR reaction products resolved by 2% agarose gel electrophoresis. The gel was Southern blotted and hybridized with probes specific for (B) DSC1; (C) DSC2; (D) DSC3. Although no band is visible in the trachea lane of the DSC1 probed blot, a band appeared after prolonged exposure. Tissues probed as legend to Fig. 5. The position of the 492-bp marker is indicated at the left hand side of each photograph. Because DSC1 is very much more abundant in *E* and *T*, these lanes in *B* have been exposed for a shorter time than the other DSC1 lanes. The smallest band in lane *K* of the ethidium bromide-stained gel (*A*) is a non-specific product since it is not recognized by any of the desmocollin probes.

and liver. A DSC2 product was visible in all epithelial tissues and cardiac muscle, and a DSC3-size product in epidermis, tongue, oesophagus, trachea, rumen, and kidney (Fig. 6 *A*). Blotting with specific probes at high stringency (Fig. 6, *B*, *C*, and *D*) confirmed the identity of these products, with the exception of the DSC3-size product in kidney which was not DSC3 specific. Surprisingly, a DSC1 product was weakly detected by Southern blotting in cardiac muscle and epithelial tissues other than epidermis, tongue, intestine, and liver (Fig. 6 *B*). Thus both DSC1 and DSC2 are ubiquitously expressed in epithelial tissues, but DSC3 appears restricted to stratified epithelia and trachea.

#### *Differentiation-related Expression Patterns of the Three Desmocollin Genes in Epidermis*

The expression patterns of the three DSC genes in bovine nasal epidermis were examined by *in situ* hybridization using  $^{35}$ S-labeled RNA probes. DSC1 showed uniform expression suprabasally, in the spinous layer (Fig. 7 *c*), with the upper limit of expression near the spinous/granular layer border. It was not detectable in the basal, granular, or cornified layers. At the bases of the rete ridges DSC1 expression was also undetectable in the first few suprabasal layers (~5–10), but at the sides of the rete ridges expression began in the first suprabasal layer (Fig. 7, *c* and *d*).

DSC2 expression also appeared to be suprabasal (Fig. 7 *e*), appearing strongest in several cell layers near the bases of the deep rete ridges. The upper limit of this strong DSC2 expression corresponded approximately to the lower limit found for DSC1.

Reduced expression then appeared to persist in the rest of the spinous layer and to cease at the border with the granular layer. At the sides of the rete ridges DSC2 expression appeared to begin in the first suprabasal layer. DSC2 expression seemed to be weak or absent from the basal layer, especially at the bases of the rete ridges (Fig. 7, *e* and *f*).

In contrast, DSC3 appeared to be most strongly expressed in the basal layer (Fig. 7, *g* and *h*). Expression appeared to extend into the first few suprabasal cell layers in the bases of the deep rete ridges but possibly to be confined to the basal cell layer at the sides of the rete ridges. The upper limit was not clearly defined, DSC3 expression apparently decreasing gradually in the lower third of the spinous layer.

#### *Expression of Dsc1 Glycoprotein Demonstrated by Specific Monoclonal Antibody*

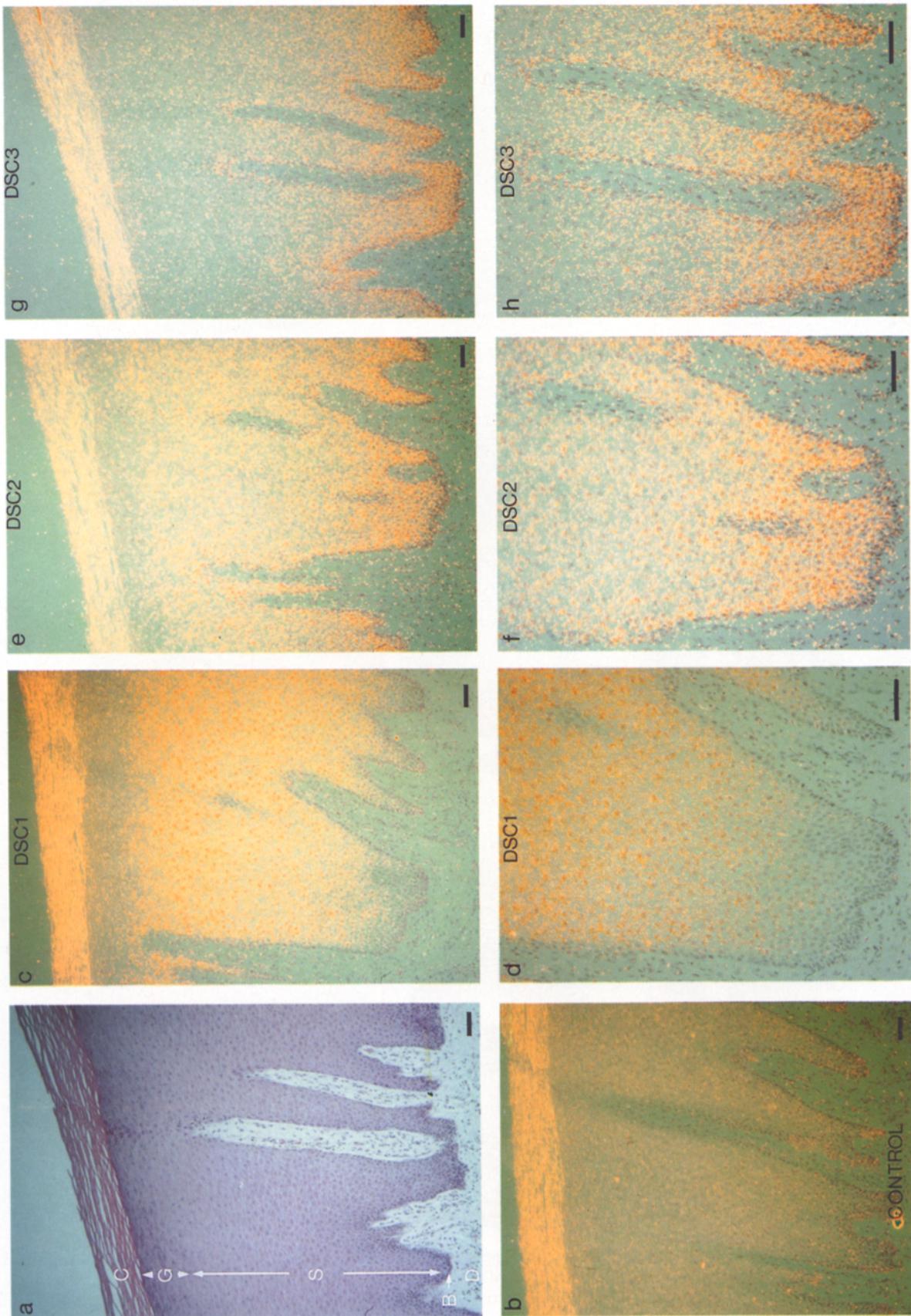
Monoclonal antibody 07-4D reacts with a 19-kD proteolytic fragment from the NH<sub>2</sub>-terminal region of desmocollins and binds to the desmosomal intercellular space by immunoelectron microscopy (Holton et al., 1990). Staining sections of bovine epidermis with 07-4D revealed patterns highly reminiscent of DSC1 expression shown by *in situ* hybridization (Fig. 7). Thus 07-4D labeling was absent from the basal layer and from several (5–10) suprabasal layers at the bases of deep rete ridges (Fig. 8, *c* and *d*). In epidermis surrounding dermal papillae, 07-4D labeling excluded only the basal layer, but was present at the interface between basal and immediately suprabasal cells (Fig. 8, *e* and *f*). By contrast, labeling of epidermis for desmoplakins (monoclonal antibody 11-5F) and a monoclonal antibody (52-3D) which labels desmocollins in many tissues (Garrod and Collins, 1992) extended throughout the living layers of the epidermis, including the basal layer (Fig. 8, *a* and *b*). Thus 07-4D may be specific for Dsc1 glycoprotein.

To confirm Dsc1 specificity 07-4D was immunoblotted with Dsc1 and Dsc2 fusion proteins expressed in *E. coli*. The antibody reacted with Dsc1 but not Dsc2 (Fig. 9). Reactivity with Dsc3 could not be tested by immunoblotting because NH<sub>2</sub>-terminal sequence was not available. However, since 07-4D does not react with the basal cell layers of either epidermis or tongue, Dsc3 reactivity seems unlikely. We conclude that 07-4D is Dsc1-specific and that its staining pattern demonstrates correspondence between the expression of Dsc1 glycoprotein and DSC1 mRNA in basal epidermis.

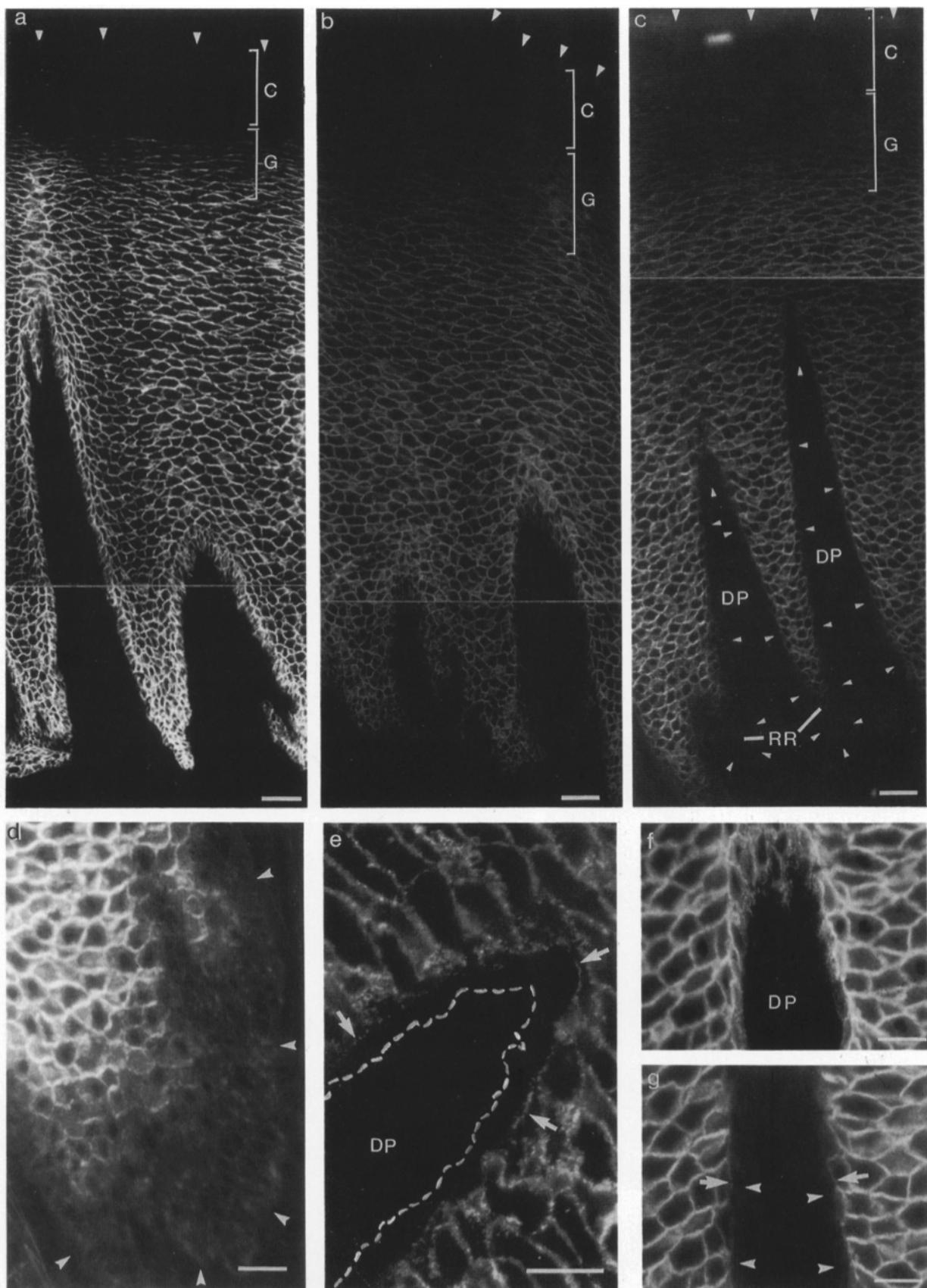
#### *Dsc1 Shows Skin-Type Differentiation in Tongue Epithelia*

The “skin”-type keratins 1 and 10, normally associated with terminally differentiating epidermal keratinocytes, are expressed in tongue epithelium in a pattern restricted to the posterior compartment of filiform papillae (Rentrop et al., 1986; Dhouailly et al., 1989). Since DSC1 expression in epidermis is associated with suprabasal cells, presumably committed to terminal differentiation, it was of interest to determine the expression of DSC1 in tongue epithelium.

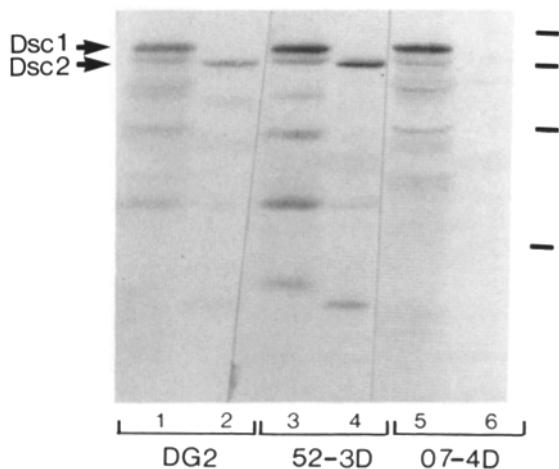
Staining of tongue epithelium with monoclonal antibodies to desmoplakin (11-5F; Fig. 10, *a* and *b*) and desmocollins (52-3D; not shown) indicates expression of these proteins throughout the epithelium, including the posterior compartment of the filiform papilla. By contrast, staining with Dsc1-specific monoclonal antibody 07-4D shows that the protein is restricted to the posterior compartment of the filiform papilla (Fig. 10 *c*). *In situ* hybridization revealed that DSC1 mRNA expression is likewise restricted to the posterior filiform papilla (Fig. 10 *d*). Thus the expression pattern of DSC1 appears to correspond closely to that previously described for keratins 1 and 10. Fluorescent staining of a wide



**Figure 7.** Expression of DSC mRNAs in nasal epidermis. (a) Haematoxylin and eosin stained section; (b-h) *In situ* hybridizations; (b) DSC1 sense control, showing interference caused by light refraction through the cornified layer under dark field; (c and d) DSC1 anti-sense probe; (e and f) DSC2 anti-sense probe; (g and h) DSC3 anti-sense probe. Higher magnifications of regions of c, e, and g are shown in d, f, and h. D, dermis; C, cornified layer; G, granular layer; S, spinous layer; B, basal layer. Bar, 50 µm.



**Figure 8.** Staining of nasal epidermis by DSC-1 specific monoclonal antibody 07-4D. (a-c) Sections showing full thickness of epidermis stained for desmoplakins (McAb 11-5F) (a), desmocollins (McAb 52-3D), (b) and McAb 07-4D (c). In a and b both antibodies stain the entire epidermis from the basal to the granular layer [G], but not the cornified layer [C]. In c, 07-4D staining begins in the first suprabasal layer at the sides of the dermal papillae [DP] but several layers above the basal layer at the tips of the rete ridges [RR]. (Small arrowheads



**Figure 9.** Mouse monoclonal antibody 07-4D is specific for Dsc1. Western blot of bacterial cell lysates of IPTG induced pUC18-CN35-XbaI (*Dsc1*) and pUC18-BMDCT2-Sac/Hind (*Dsc2*) transformants; lanes 1, 3, and 5, pUC18-CN35-XbaI transformed cells; lanes 2, 4, and 6 pUC18-BMDCT2-Sac/Hind transformed cells. The specific anti-desmocollin antibodies used are indicated at the bottom of the figure. DG2 is a guinea-pig antiserum and 52-3D a mouse monoclonal antibody to bovine desmocollins. Both show wide tissue reactivity. Note that both these antibodies react with both fusion proteins (lanes 1-4). 07-4D reacts only with the *Dsc1* fusion protein (lanes 5 and 6). The positions of the *Dsc1* and *Dsc2* fusion proteins are indicated (arrows). Lower molecular weight bands represent breakdown products of the fusion proteins. Markers 116, 97, 66, and 45 kD.

range of other epithelia with 07-4D indicates an absence of *Dsc1* protein expression (results not shown).

#### ***DSC2 and 3 Show Differentiation-related Expression in Other Stratified Epithelia***

*In situ* hybridization with DSC-specific probes on interpapillary epithelium of tongue, and oesophageal and ruminal epithelia, showed quite characteristic patterns. In oesophagus (Fig. 11) and rumen (results not shown) there was no detectable expression of DSC1. DSC2 showed strong expression in a clearly defined band beginning above the basal layer and extending to approximately half the thickness of the epithelium. DSC2 expression appeared much weaker in, or possibly absent from, basal layers. By contrast DSC3 expression was strongly basal extending into two or three suprabasal layers. Thus DSC2 and DSC3 have very similar and characteristic expression patterns in all stratified epithelia. Staining of these oesophageal and ruminal epithelia with monoclonal antibodies 11-5F and 52-3D (results not shown) indicate that desmosomal components and desmosomes are expressed throughout the thickness of the epithelium even

though desmocollin mRNAs were restricted to the basal half.

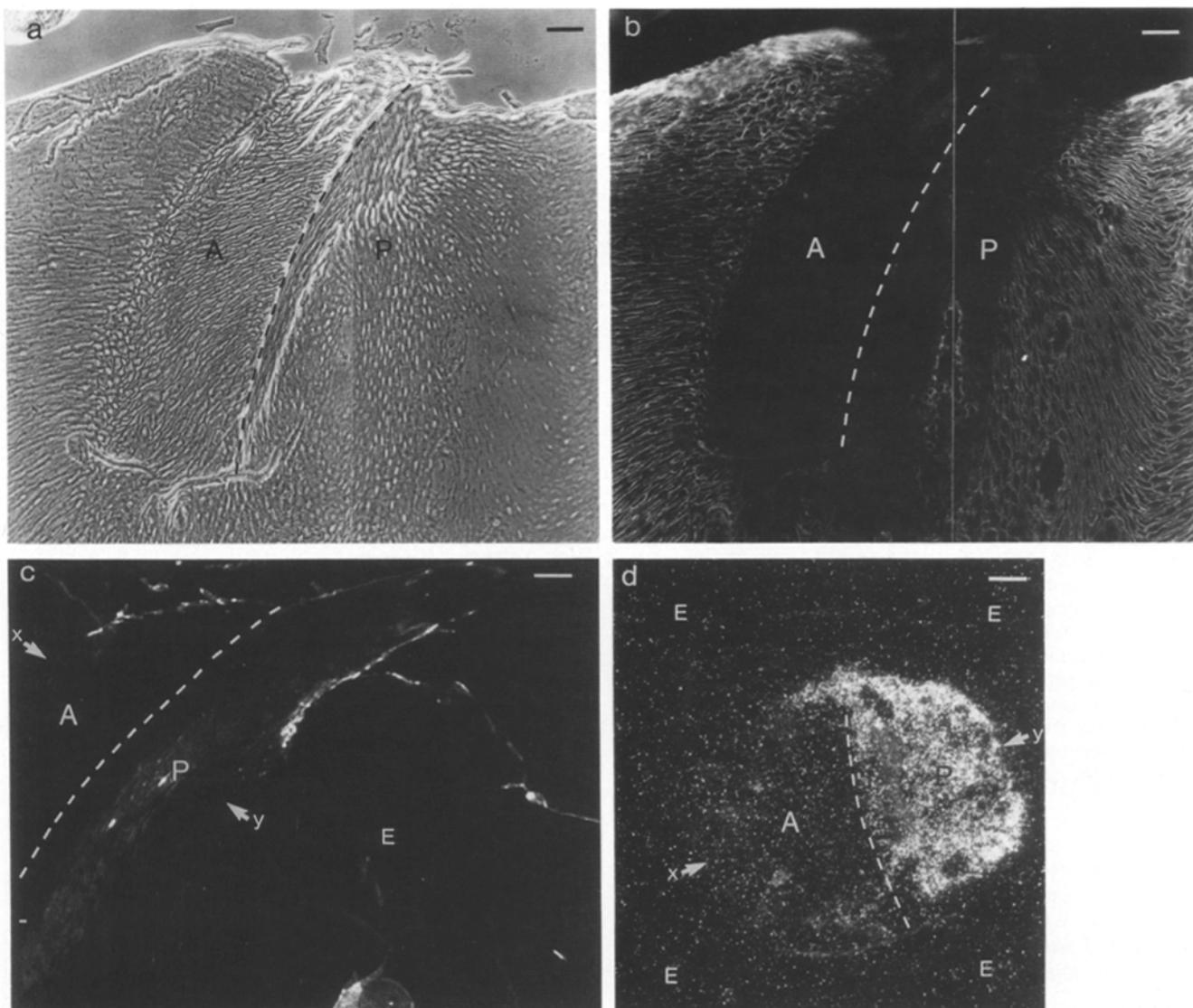
#### **Discussion**

The data presented in this paper provide a standard for the nomenclature of the desmocollin genes and proteins proposed recently by Buxton et al. (1993). We demonstrate the existence of third bovine desmocollin gene. Thus there are at least three bovine desmocollin genes, DSC1, DSC2, and DSC3. Each gives rise, by alternative splicing, to two proteins, the a and b forms, that differ in the size of their cytoplasmic domains (Collins et al., 1991; Koch et al., 1992; and present data). Our data tentatively suggest that DSC3 may be the homologue of human DGII/III. Although DSC1 is clearly distinct from the other desmocollin isoforms, DSC2, DSC3 and DGII/III are closely similar in percentage sequence identity. Other criteria are therefore required to distinguish between them. DSC3 resembles DGII/III in two respects: (a) DGII/III, like Dsc3, shows two partially conserved repeats of a 9-amino acid sequence in the cytoplasmic domain, whereas Dsc2 shows three such repeats (Fig. 4). (b) *In situ* hybridization appears to show that DGII/III is most strongly expressed in the basal layer of epidermis (Arnemann et al., 1993; Theis et al., 1993), like Dsc3, but unlike Dsc2 (Fig. 7).

Our data reveal several novel aspects of desmocollin expression. Thus the three desmocollin genes show quite distinct and characteristic expression in relation to types of epithelial differentiation and to the likely proliferative capacity of epithelial cells. DSC1 shows its major expression in relation to terminal differentiation in epidermis and tongue. Its sharply localized expression in tongue papillae is particularly informative. Adjacent regions of filiform papillae may be classified into oesophageal-, skin-, and hair-type differentiation on the basis of keratin type expression (Dhouailly et al., 1989). DSC1 expression in these papillae clearly corresponds to the region of expression of keratins type 1 and 10, the skin-type keratins (Dhouailly et al., 1989). The strictly suprabasal expression of DSC1 in epidermis also strongly resembles that of keratins 1 and 10 (Woodcock-Mitchell et al., 1982). We conclude that DSC1 is predominantly a skin-type desmocollin.

The monoclonal 07-4D is the first properly characterized desmocollin isoform-specific antibody. It labels epidermis from the first suprabasal layer, including the interface with basal cells. This reactivity corresponds precisely with that reported previously for mouse polyclonal anti-desmocollin antibodies on human and bovine epidermis (Parrish et al., 1986). The latter antibodies may therefore have been DSC1-specific. It is of some concern that the labeling pattern obtained with 07-4D does not correspond exactly with that found by King et al. (1993b) when they stained human

indicate the basement membrane; large arrowheads the apical surface of the epidermis.) (Bars, 50 µm). (d-g) High power fluorescence micrographs. (d) Base of rete ridge showing that 07-4D staining begins several layers from the tip (arrowheads indicate basement membrane). (e) Transverse section of dermal papilla showing absence of 07-4D staining from the basal layer. Position of basal lamina, indicated by dashed line, determined by double staining for laminin (not shown). Note that basal surfaces of the immediately suprabasal cells stain with the antibody (arrows). (f and g) Tip of dermal papilla double stained with guinea-pig antidesmoplakin antibody (f) and 07-4D (g). The basal layer is clearly stained in f but not in g. (Arrows—07-4D staining of basal surface of first suprabasal layer; arrowheads, basement membrane). Bars, 25 µm.



**Figure 10.** DSC1 expression in filiform papillae of tongue epithelium. (a) Section of tongue epithelium visualized by phase contrast microscopy showing filiform papilla. The tip of the papilla has broken off during sectioning. (b) Field as *a* stained with monoclonal antibody 11-5F and visualized by fluorescence microscopy. (c) Section of filiform papilla stained with 07-4D and visualized by confocal microscopy showing that staining is confined to the posterior part of the papilla. (d) *In situ* hybridization with DSC1-specific probe on transverse section of a filiform papilla at approximately the level indicated (*x-y*) in *c*. The dashed line indicates the boundary between the anterior (*A*) and posterior (*P*) regions. *E*, interpapillary epithelium. Bars, 50  $\mu$ m.

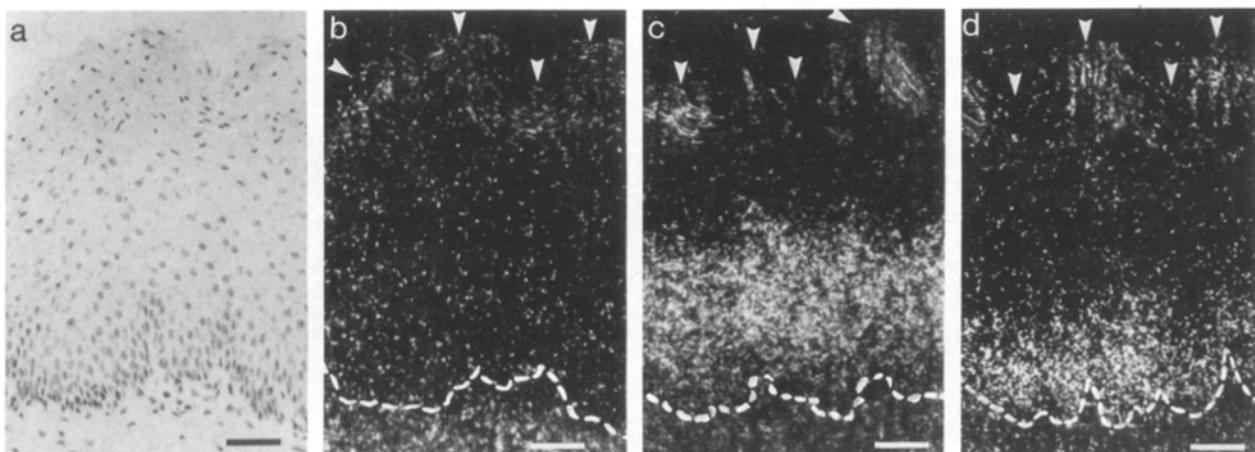
epidermis with a monoclonal antibody believed, but not proven, to be specific for human DSC1. The significance of this discrepancy is not clear at present.

Neither 07-4D staining nor Northern blotting revealed DSC1 expression in tissues other than epidermis and tongue. It was therefore surprising to find a low level of ubiquitous epithelial DSC1 expression by RT-PCR. These results are unlikely to be caused by cross-contamination between samples because careful controls were carried out. Since the level of expression is so low, we conclude that it may have no functional significance.

DSC2 is ubiquitously expressed in epithelia and cardiac muscle. Its expression in a small sample of simple and stratified epithelia was previously indicated by Koch et al.

(1992). While we cannot rule out its presence in the basal layer, DSC2 expression in stratified epithelia appears to be predominantly suprabasal, becoming strong immediately above the basal layer and extending evenly to a level about one half to two thirds of the epithelial thickness. Thus *Dsc2* mRNA expression and therefore glycoprotein synthesis cease substantially below the level to which desmosomes are present as indicated by fluorescent antibody staining for desmoplakins (not shown). In these epithelia, DSC2 expression therefore seems to be associated with cells that have begun the terminal differentiative process.

By contrast, DSC3 is strongly expressed in the basal layers of all four stratified epithelia studied. Expression then fades in the immediately suprabasal layers. Moreover, DSC3 ap-



**Figure 11.** Expression of DSC mRNAs in oesophageal epithelium. (a) Haematoxylin-stained section; (b-d) *in situ* hybridizations; (b) DSC1 anti-sense probe; (c) DSC2 antisense probe; (d) DSC3 anti-sense probe. In b-d, the lower edge of the basal layer is marked by a dashed line, and the upper surface of the epithelium by arrowheads. Bar, 50  $\mu$ m.

pears to be restricted to stratified epithelia and the pseudostratified epithelium of trachea. DSC3 is therefore strongly associated with generative cell layers of stratified epithelia.

A relationship between desmocollin isoform expression and cell proliferation is further suggested because there is a very striking resemblance between the expression patterns of desmocollins and the distribution of stem cells (SC), transit amplifying (TA) cells, and terminally differentiated (TD) cells in ridged epidermis as described for monkey palmar epithelium by Lavker and Sun (1983) (See also Cotsarelis et al., 1989). These authors propose that slowly cycling SC are located at the tips of epithelial rete ridges in the form of "non-serrated" basal cells. SC give rise to a population of rapidly cycling TA cells in the suprabasal layers principally at the bottom of the rete ridges. TA cells then generate non-cycling TD cells. Comparison of our *in situ* hybridization data with the thymidine incorporation data of Lavker and Sun (1983) shows that DSC3 is associated with basal cells among which are SC, the peak region of DSC2 expression corresponds with the location of TA cells, while DSC1 is associated with TD cells. We conclude that there is correspondence between the major type of desmocollin isoforms expressed by epidermal cells and their proliferative capacity.

We have shown recently that a number of epithelial cell types, including basal keratinocytes, retain desmosomes, and other types of junctions during cell division (Baker and Garrod, 1993). The presence of different desmocollin isoforms in cycling and non-cycling cells is, therefore, unlikely to be associated with facilitating junctional breakdown during division. A more intriguing possibility is that the different isoforms may in some way signal the state of differentiation and therefore proliferative status to the cells. It is now well-established that adhesion molecules transduce signals across the cell membrane both inwards and outwards, and that such signals can modulate cell behavior, proliferation, and gene expression (see for example Hynes, 1992). Evidence is beginning to emerge that desmosome respond to both intracellular and extracellular signals (Garrod, 1993).

It may be that desmosomal glycoproteins can also transduce environmental signals.

Classical cadherins mediate cell sorting and positioning in morphogenesis (for review see Takeichi, 1988, 1990; Geiger and Ayalon, 1992). In the developing mouse epidermis E- and P-cadherin show differential expression (Hirai et al., 1989) and E-cadherin has been implicated in the control of stratification in keratinocytes (Wheelock and Jensen, 1992). We suggest that the desmosomal cadherins (desmocollins and desmogleins) may contribute to epidermal cell positioning, i.e., stratification, by mediating differential adhesive ness between cells that express different isoforms. Ultrastructural studies have shown that both size and number of desmosomes increases between basal and suprabasal epider mal layers (Skerrow et al., 1989), also suggesting that desmosomal adhesion changes with stratification.

A complicating factor is that regions of expression of different desmocollin isoforms clearly overlap, so that individual cells at different levels in an epithelium express different combinations of the desmocollins, rather than distinct isoforms. Does this mean that a cell may possess individual desmosomes with distinct desmosomal isoforms, or that individual desmosomes contain mixtures of glycoprotein isoforms? This question may be solved by immunoelectron microscopy given the future availability of further isoform-specific antibodies. In either case it seems likely that variations in desmosomal adhesion between different cell layers may be quite subtle, providing a gradual or graded change in desmosomal adhesion at different levels.

Our *in situ* hybridization results broadly resemble the pattern described for expression of three *desmoglein* genes in human epidermis by Arnemann et al. (1993). The expression patterns of the two human *desmocollin* genes described by Arnemann et al. (1993) do not obviously overlap, DGII/III being basal and DGIV/V in the high suprabasal region. Thus it seems likely that a third human desmocollin isoform remains to be discovered. By contrast with the glycoproteins, there is no evidence that the major desmosomal proteins,

desmoplakins and plakoglobin, show stratification-related expression of different isoforms. This suggests that the desmosomal glycoproteins rather than the proteins are determinants of any differential function related to epithelial differentiation and stratification.

We thank Drs. M. Dmochowski, T. Hashimoto, and Professor T. Nishikawa for their generous gift of pUC18-CN35-XbaI; and Professor W. W. Franke for clone BMDCT2-DC4.1. We would also like to thank Drs. J. A. Davies, J. Lorimer, A. V. North, and C. H. Streuli for helpful discussions and criticism of the manuscript.

This work was supported by the Cancer Research Campaign, the Medical Research Council, and the Agricultural and Food Research Council.

Received for publication 23 August 1993 and in revised form 4 April 1994.

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