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Complexity and expression patterns of the desmosomal cadherins

(desmosomes/cell junctions/cell adhesion/desmoglein/desmocollin)

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ABSTRACT **Desmosomes are intercellular junctions that contain two major kinds of transmembrane glycoproteins, desmoglein and desmocollins I and II, involved in cell–cell adhesion. Recent sequence analyses have shown that both desmosomal glycoproteins belong to the larger cadherin family of cell adhesion molecules, in which they represent two different subgroups characterized by their specific sequence and topogenesis. In analyses of cDNA sequences and Northern blot experiments we have now found that both desmoglein and desmocollins are not unique gene products but occur in different subtypes produced from different genes. Comparison of the complete amino acid sequences of type 1 and type 2 desmocollins and of two desmoglein subtypes shows considerable divergence. While the desmoglein genes can be differentially expressed in different cell types, both type 1 and type 2 desmocollins can coexist in the same cells of certain stratified epithelia as shown by *in situ* hybridization. We conclude that the cadherin composition of desmosomes is much more complex than assumed and can differ in the various epithelia.**

Both major kinds of intercellular adhering junctions bearing a dense cytoplasmic plaque have recently been shown to contain glycoproteins of the larger family of cadherins, a group of cell adhesion molecules (CAMs) characterized by a single transmembrane segment flanked by an extended extracellular portion with four repeating units of ≈110 amino acids each and a cytoplasmic tail (Fig. 1; refs. 1, 6, and 7). The "adhering junctions," including the zonula adherens, the fascia adherens, and the puncta adherentia, the plaque of which anchors actin microfilaments, have been shown to contain E-cadherin (uvomorulin, L-CAM; ref. 8; for reviews see refs. 9–11). The desmosomes (maculae adherentes), whose plaques provide attachment sites for intermediate filaments, have been shown to contain two different desmosome-specific subspecies of cadherins. Desmocollins I and II, two closely related variants generated by alternative splicing of transcripts from the same gene, are similar in size (calculated molecular weights of 85,000 and 79,000 for the bovine muzzle epithelial proteins) to E-cadherin and also have a similar carboxyl terminus (2–4, 12), whereas desmoglein is a larger polypeptide (calculated molecular weight of 106,000) with a cytoplasmic extension exceeding that of desmocollin I by 270 amino acid residues (1, 6, 13–15). This has already made clear that the same epithelial cell contains different cadherins [E-cadherin, desmoglein, desmocollin(s)] whose specific contributions to homotypic cell sorting and binding have yet to be determined.

Evidence accumulated so far has indicated that one desmoglein and one desmocollin I and II exist in a given species. Observations of heterogeneity and cell type-related differences of electrophoretic mobility and antibody reactivity (e.g., refs. 16–22) could be ascribed to possible different forms of modification, notably glycosylation, of proteolytic trimming (see, however, ref. 22) or of alternative splicing (2). In the course of cDNA cloning studies of desmosomal proteins we have now recognized that genotypically different subtypes of both desmoglein and desmocollins exist in the same species and can be expressed in complex patterns.†

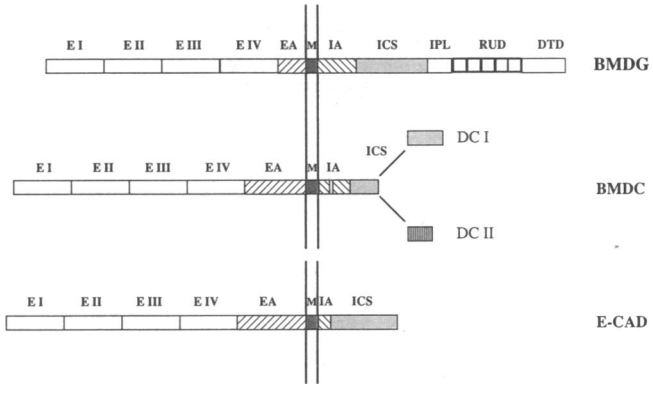

FIG. 1. Homology and functional correspondence of individual domains in bovine muzzle epithelial desmoglein (BMDG; ref. 1), desmocollin(s) (BMDC, with two subtypes differing in their carboxyl terminal domain, apparently generated by alternative splicing: DC I and DC II; refs. 2–4), and mouse E-cadherin (E-CAD; ref. 5). The domains are designated E I–IV (extracellular repeating elements), EA (extracellular anchoring domain), M (membrane-spanning domain), IA (intracellular anchoring domain), ICS (intracellular cadherin-type segment), IPL (intracellular proline-rich linker region), RUD (domain containing five repeating units), and DTD (desmoglein-specific terminal domain). The membrane is symbolized by vertical lines. The interruption of the IA domain of BMDC is due to an insertion of 32 amino acids that is unique to desmocollin.

MATERIALS AND METHODS

Screening, Cloning, and Sequencing of cDNA. A λZAPII cDNA expression library constructed from bovine muzzle epithelial poly(A)$^+$ RNA ("R-lib"; ref. 1) was screened with guinea pig antibodies against bovine desmocollins I and II (for methods of screening and sequencing, see ref. 3). The various cDNA clones isolated and sequenced could be divided into two groups: one contained clones encoding the typical muzzle epithelial desmocollin(s) (3), the other contained insertions homologous to—but not identical with—the previously published desmocollin sequences (2–4, 12). One clone from the second group, termed BMDCT2-DC11.2 (data not shown), was chosen to rescreen the library. Among the various clones isolated, the clone with the longest cDNA

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Abbreviation: CAM, cell adhesion molecule.

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†The sequence reported in this paper has been deposited in the EMBL/GenBank data base (accession no. M81190).

insertion obtained (BMDCT2-DC4.1) was chosen for further analysis.

Polymerase Chain Reaction (PCR). Using total RNAs of various bovine tissues and cell lines, we synthesized cDNAs (23). Oligonucleotides 373 (5′-TTGTTGGGCATAGCAT-TGCT-3′; positions 1952–1971 of BMDCT2-DC4.1) and 374 (5′-TAGTCTTGAGCTTGCATATG-3′; complementary to positions 2472–2491 of BMDCT2-DC4.1) were used to amplify type 2 desmocollin cDNAs (for methods see ref. 1). Two DNA fragments of approximately 0.54 kilobases (kb) and 0.49 kb amplified from cDNA of BMGE+H cells were subcloned in pBluescript (Stratagene) and sequenced (clones DCPCR9 and DCPCR15). The cDNA sequences of both PCR clones were found to be basically identical to BMDCT2-DC4.1, except for a stretch of 46 base pairs (bp) present in DCPCR15 and BMDCT2-DC4.1 but not in DCPCR9.

RNA Preparation and Northern Blot Analysis. Methods used for the isolation of RNA from various tissues and cell lines (MDBK, ref. 24; BMGE+H, ref. 25; calf lens cells, ref. 26; HaCat, ref. 27) and for Northern blot hybridization (28, 29) were as described. RNA blots were probed with ^{32}P-labeled antisense RNA derived from clones BMDCT2-

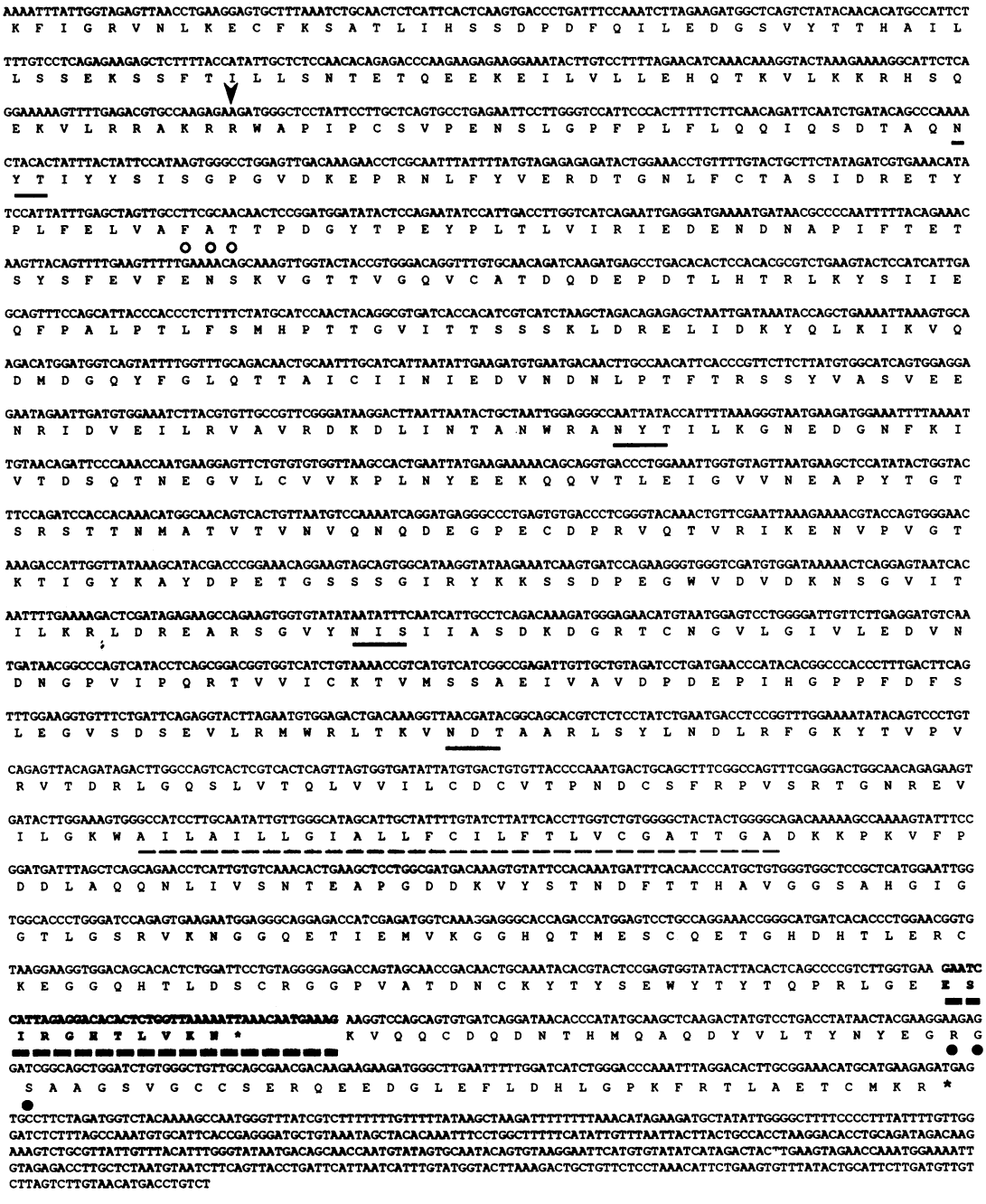

FIG. 2. cDNA (upper numbers) and deduced amino acid (lower numbers, one-letter code) sequences of bovine muzzle epithelial type 2 desmocollin(s) as determined from clone BMDCT2-DC4.1. The sequence encodes the entire mature protein and most of the precursor protein but does not fully extend to the amino terminus of the unprocessed polypeptide precursor. The arrowhead indicates the probable cleavage site for the generation of the mature BMDCT2 polypeptide (see also ref. 3). Possible N-glycosylation sites are underlined. Instead of the sequence HAV of E-cadherin, BMDCT2 contains the motif FAT (open circles). The single candidate sequence sufficiently long for a transmembrane domain is underscored by the thin interrupted line. The bold interrupted line denotes a 46-bp insertion found only in some of the clones (shown here is BMDCT2-DC4.1), encoding 11 amino acids and an in-frame stop codon. A single possible serine phosphorylation site (RGS) is indicated by filled circles. Stop codons are represented by stars. In two other independently obtained cDNA clones we noted two nucleotide changes: A → C in position 791, resulting in a change from lysine to glutamine, and G → A in position 999, replacing an arginine by a glutamine.

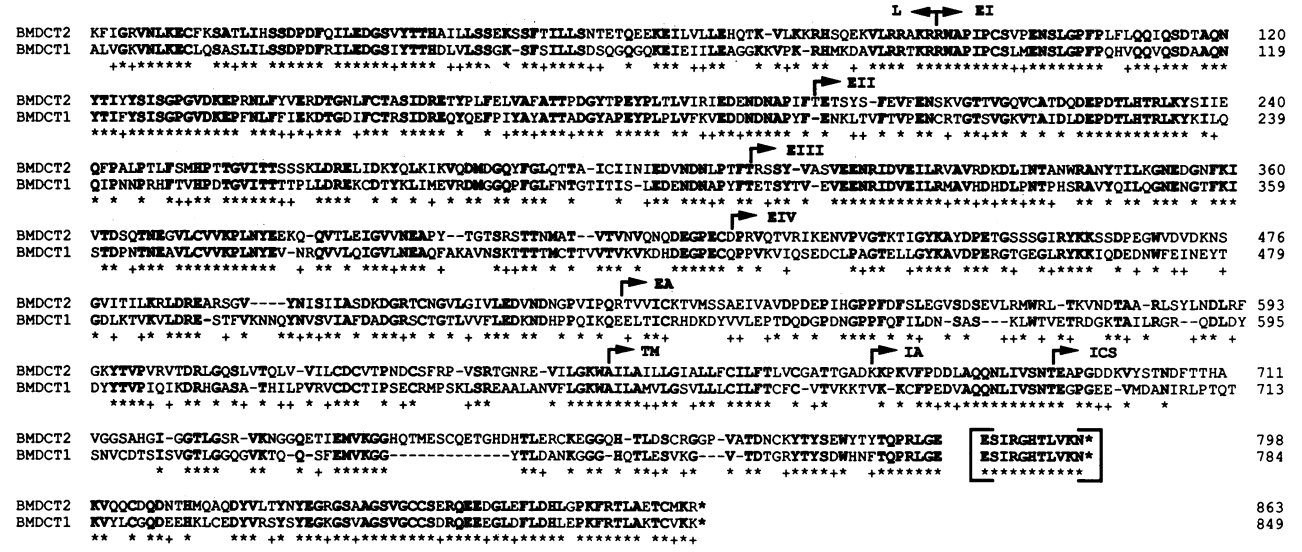

FIG. 3. Different desmocollins in the same species shown by alignment of the amino acid sequences of genotypically different desmocollins existing in the same tissue: bovine muzzle epithelial type 1 (BMDCT1; positions 1–88 are from the precursor sequence in ref. 2) and type 2 (BMDCT2) desmocollin. Domains of the mature proteins are indicated with arrows: EI–EIV, extracellular repeating units; EA, extracellular anchoring domain; TM, transmembrane domain; IA, intracellular anchoring domain; ICS, intracellular cadherin-type segment; L, leader sequence of the precursor. Identical amino acids (boldface) are indicated by stars, and conservative exchanges (i.e., amino acids with similar chemical properties) by plus signs. The 11 amino acids that represent the carboxyl terminal end of type 1 and type 2 desmocollin II are indicated by brackets (compare with Fig. 1); these are not present in type 1/type 2 desmocollin I, whose carboxyl terminus is indicated by stars.

DC11.2 (positions 1349–2488 of BMDCT2-DC4.1), BDC-7.5 (3), pHDGI (6), and HDGC-pBXF (6).

***In Situ* Hybridization.** An antisense cRNA probe from type 2 desmocollin (positions 1952–2491 of BMDCT2-DC4.1 cDNA) was synthesized using [α-[^{35}S]thio]CTP (Amersham) according to standard procedures. The labeled cRNA was precipitated, dissolved in 0.1 M NaOH, hydrolyzed for 10 min on ice, precipitated, and dissolved in hybridization buffer (50% formamide/0.3 M NaCl/5 mM EDTA/10 mM dithiothreitol/1× Denhardt's solution/0.05% yeast tRNA/10% dextran sulfate/20 mM sodium phosphate buffer/20 mM Tris·HCl, pH 6.8).

The preparation of tissue slices, the hybridization conditions (cRNA at 100 ng/ml), and the washing procedure were as described (30), except that the tissue slices were incubated for 30 min at 37°C with proteinase K (1 μg/ml) and, after hybridization, were treated with RNase A (20 μg/ml) for 30 min at 37°C. All washing steps were done at 52°C.

Southern Blots. Genomic DNA from calf thymus was digested with various restriction endonucleases, and the fragments obtained were electrophoresed in 1% agarose gels and transferred to Biodyne A transfer membranes (Pall).

Type 1 and type 2 desmocollin-specific probes were generated by PCR (1) using bovine genomic DNA as a template and the following primers: 5′-ACCCTGGAATCTGTTAAGGGAGTCACGGA-3′ (specific for type 1), 5′-GGAGGACCAGTAGCAACCGACAACT-3′ (specific for type 2), and 5′-TTAACCAGAGTGTGTCCTCTAATG-3′ (both types 1 and 2). The amplified fragments containing intron sequences were cloned and sequenced (data not shown). *Eco*RI–*Nsi* I fragments of the cloned PCR products were used to synthesize ^{32}P-labeled random-primed probes.

Hybridization and washing procedures were performed according to the manufacturer's protocol (Pall).

RESULTS

Genetically Distinct Subtypes of Desmocollins. In the course of Northern blot hybridization and nucleotide sequencing we have noticed that at least two major groups of desmocollin cDNAs isolated from the same tissue—i.e., bovine muzzle epithelium—can be distinguished: (*i*) type 1 desmocollin with its two subforms generated by alternative splicing, termed desmocollins I and II (for sequence analysis see refs. 2–4, 12), and (*ii*) a type 2 desmocollin, which again appears in two subforms, also termed I and II. The complete amino acid sequence of the mature type 2 polypeptide, as deduced from the nucleotide sequence of one of the cDNA clones, is shown in Fig. 2, including a large proportion of the precursor polypeptide.

The comparison of the two types of desmocollins is shown in Fig. 3. Both are of similar size (the calculated molecular weight of the 761 amino acid residues of the processed polypeptide of type 1 desmocollin I is 85,170, and that of type 2 desmocollin I is 85,624 for 774 amino acids), both occur in two different subforms differing in the carboxyl terminal domain (see below), and both have very similar isoelectric points. However, they differ considerably in their sequences: only 46% of the amino acids are identical in the corresponding positions, and the total homology is 69% (Fig. 3), being highest in the extracellular domain E I (87%; 72% identity;

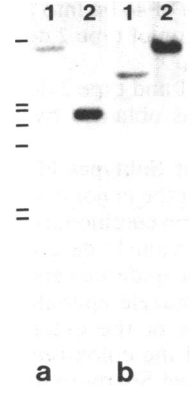

FIG. 4. Southern blot analysis of genomic bovine DNA digested with *Hin*dIII (lanes 1) or *Bam*HI (lanes 2) and hybridized with a probe specific for the novel, type 2 desmocollin (*a*) or with a probe specific for type 1 desmocollin (*b*). Bars at left indicate mobilities of phage λ DNA fragments obtained by digestion with *Eco*RI and *Hin*dIII.

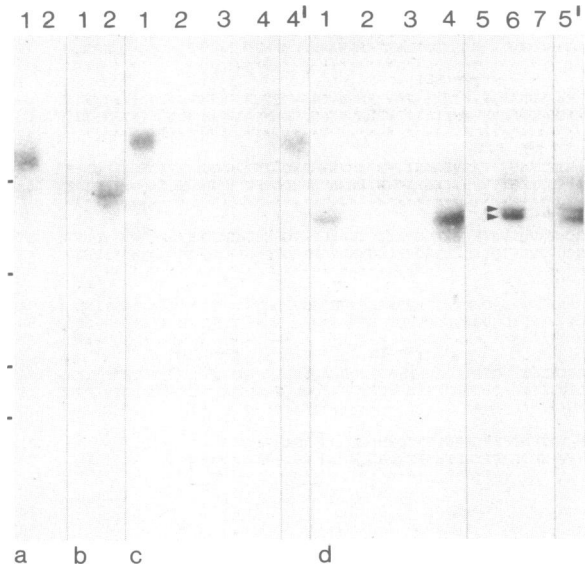

FIG. 5. Tissue and cell-type specificity of desmoglein (*a–c*) and desmocollin (*d*) expression as seen from Northern blot analysis of total RNA (20 μg per lane). (*a*) RNAs from human keratinocyte-derived cell line HaCaT (lane 1) and human epidermis (lane 2) probed with clone HDGC-pBXF (21). (*b*) Same RNAs as in *a* but probed with pHDGI (21). (*c*) RNAs from bovine muzzle epithelium (lane 1), calf lens cells (lane 2), bovine heart (lane 3), and bovine tongue mucosa (lane 4) probed with clone BDC 7-5 (1). Lane 4′ is a longer exposure of lane 4. (*d*) Same RNAs (lanes 1–4) as in *c* plus RNA from cultured bovine kidney (MDBK) cells (lane 5; lane 5′ is longer exposure of lane 5), cultured bovine mammary gland epithelial cells (BMGE+H; lane 6), and calf lens cells (lane 7) probed with BM-DCT2-DC11.2. Molecular sizes of the specific hybridized bands are approximately 5.5 kb (human colon-type desmoglein mRNA, HDGC-pBXF), 4.6 kb (human keratinocyte-type desmoglein, pH-DGI), 6 kb [bovine muzzle epithelial desmocollin(s), BDC 7-5], 4 kb and 4.2 kb [arrowheads in *d*, bovine muzzle epithelial type 2 desmocollin(s), BMDCT2-DC11.2]. Positions of molecular size markers (16S, 18S, 23S, and 28S rRNA) are indicated at left.

see Fig. 1). This means that the two types of desmocollins are similarly distant from other cadherins such as E-cadherins and desmoglein (refs. 1, 3, and 6; see also ref. 31). In addition, we have found two different clones showing two amino acid exchanges (Fig. 2), which may represent alleles.

Using PCR, we have shown that type 2 desmocollin exists, in the same tissues (bovine muzzle epithelium and tongue mucosa) and in the same cell lines (BMGE+H, MDBK), in two forms, a larger one (subform I; see Fig. 1) and a shorter one (subform II), both apparently generated by alternative splicing of a mini exon of 46 bp into the mRNA encoding the carboxyl terminal region of type 2 desmocollin resulting in a premature stop codon.

Evidence that type 1 and type 2 desmocollins are encoded by different genes was obtained by Southern blot analysis (Fig. 4).

Genetically Different Subtypes of Desmogleins. We have recently shown (6) that the major desmoglein found in human colon and cultured colon carcinoma cells is related to, but not identical with, the previously described type of desmoglein predominant in human epidermis and cultured keratinocytes as well as in bovine muzzle epithelium. Comparison of the amino acid sequences of the cytoplasmic portions of the keratinocyte-type and the colon-type desmogleins revealed only ≈29% identity, and Southern blot analysis showed that the two types of desmoglein were encoded by different genes (6).

Different Patterns of Expression of Desmoglein and Desmocollin Subtypes. Using Northern blot and PCR analyses as well as *in situ* hybridization, we studied the expression of the

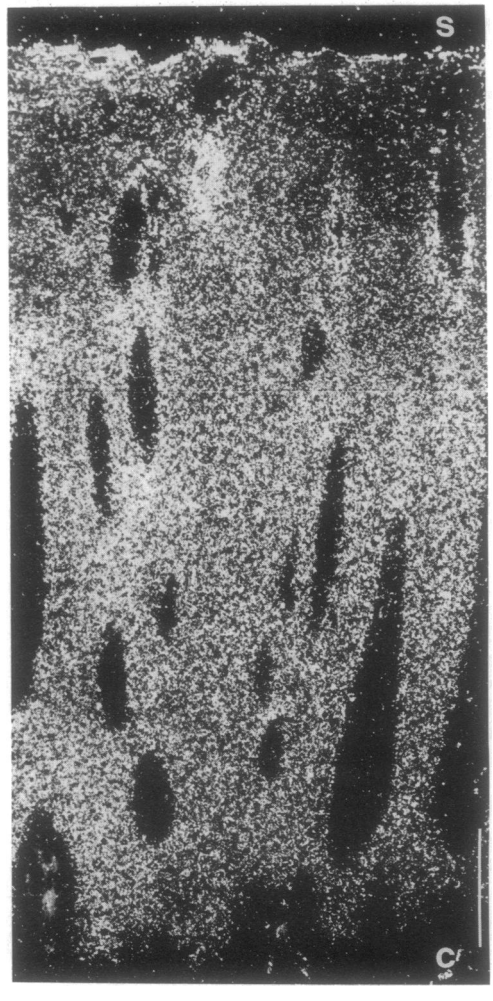

FIG. 6. *In situ* hybridization of frozen section through bovine tongue mucosa (S, surface; C, connective tissue of submucosa) probed with cRNA specific for type 2 desmocollin mRNA. Note that this mRNA occurs in all layers of this stratified epithelium. (Bar = 200 μm.)

different subtypes of desmosomal cadherins in various desmosome-containing cells and tissues. Fig. 5 *a* and *b* present Northern blot examples for desmoglein. The colon-type desmoglein was seen as a prominent mRNA of ≈5.5 kb in cultured human skin carcinoma cells (Fig. 5*a*, lane 1) but not in normal human epidermis (lane 2), whereas the ≈4.6-kb mRNA encoding the keratinocyte-type desmoglein was detected in the epidermal tissue but not in the tumor cell lines (Fig. 5*b*, lanes 1 and 2). While the colon-type desmoglein mRNA was also seen in Northern blots of several other human cell lines, including some derived from monolayer ("simple") epithelia (e.g., MCF-7, CaCo-2) and others from squamous cell carcinomas (e.g., HaCaT, A-431), the keratinocyte-type desmoglein mRNA has so far been detected only in native stratified tissues (e.g., epidermis, oral mucosa) and in cultured keratinocytes (14, 15).

A different pattern of expression was observed for the two types of desmocollins, as shown for bovine tissues and cells in Fig. 5 *c* and *d*. We found type 1 desmocollin I and II mRNAs (≈6 kb) only in muzzle epithelium (Fig. 5*c*, lane 1) and tongue mucosa (lanes 4 and 4′) but not in non-desmosome-producing tissues and cell lines, or in desmosome-containing, simple epithelium-derived cell lines such as MDBK and BMGE+H, or in heart tissue (Fig. 5*c*, lanes 2 and 3). Type 2 desmocollin mRNAs (≈4.0 and ≈4.2 kb) were also found in muzzle tissue and in tongue mucosa, though at a lower concentration (Fig. 5*d*, lanes 1 and 4) but could also be

detected in the kidney epithelium-derived cell line MDBK (Fig. 5*d*, lanes 5 and 5') and the mammary gland-derived line BMGE+H (lane 6). These mRNAs were not detected in calf lens epithelium-derived cell cultures or in myocardiac tissue (Fig. 5*d*, lanes 2, 3, and 7).

By *in situ* hybridization (Fig. 6), we found type 2 desmocollin mRNAs in all layers of tongue mucosa, in codistribution with type 1 desmocollin mRNA, whereas it was markedly enriched in the basal cell layer of muzzle epithelium (data not shown).

DISCUSSION

Our results show that neither of the two desmosomal cadherins, desmoglein and desmocollin, is a unique protein encoded by a single gene but that both desmoglein and desmocollin occur in different subtypes. Thus, the complexity and diversity of desmosome composition are much greater than hitherto thought. Moreover, our finding of cell type-specific differences of expression of desmoglein subtypes makes it clear that the desmosome is not a characteristic junctional structure with identical components in the different desmosome-containing tissues but displays differentiation-related specificities. Clearly, desmosomes of different tissues have some common (plakoglobin, desmoplakin I) as well as some cell type-specific constituents. Previously, we had noticed that desmoplakin II is absent from some and present only as a minor component in other desmosome-containing cell types (refs. 7, 18, and 24; compare ref. 32), and "band 6 protein," a desmosome-associated basic protein, has not been found in desmosomes of tissues other than stratified and complex epithelia (33).

At present, we cannot estimate the total subtype complexity of desmosomal cadherins. For example, the fact that we have not obtained positive Northern blot hybridization of cardiac RNA probed with both subtypes of desmoglein suggests that there exists another yet unknown subtype of cardiac desmoglein (for immunoblot identification of cardiac desmoglein see ref. 34). Apparently, there is a much finer specialization and tuning of desmosomal junctional coupling than so far thought.

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