Differential synthesis of type 1 and type 2 desmocollin mRNAs in human stratified epithelia

DAGMAR G. THEIS, PETER J. KOCH and WERNER W. FRANKE*

Division of Cell Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany

ABSTRACT Epithelial cells are tightly connected by various kinds of junctions, of which the desmosomes *(maculae adhaerentes)* are particularly prominent. The desmosomes are characterized by two subgroups of constitutive transmembrane glycoproteins, the desmogleins and the desmocollins, which have been identified as specific members of the larger multigene family of CAMs of the cadherin category. Following our recent observation in bovine tissues that different desmoglein and desmocollin genes can be expressed in different cell types (Koch, P.J. *et al., Proc. Natl. Acad. Sci. USA 89:* 353-357, 1992), we have now isolated cDNAs encoding human desmocollins type 1 and type 2. The complete sequence of human type 1 desmocollin has been determined and identified by its homology to the corresponding bovine gene product. Using *in situ* hybridization on sections through frozen human tissues, we show that mRNAs for type 2 desmocollin are synthesized in various stratified epithelia such as epidermis, esophagus and exocervix, whereas type 1 desmocollin was detected in appreciable amounts only in epidermis. In addition, a striking difference has been observed within the epidermis, where type 2 desmocollin mRNA can be detected in several basal layers of living cells but type 1 desmocollin mRNA is restricted to suprabasal layers. The possible functional involvement of desmocollins in the differentiation of stratified tissues is discussed and the potential value of molecular probes for desmosomal cadherins in tumor diagnosis is emphasized.

KEY WORDS: *desmosomes, junctions, cadherins, desmocollins, epithelia, terminal differentiation*

Introduction

Cell-cell recognition, sorting and coupling — and thus tissue formation — involve a diversity of cell adhesion molecules (CAMs) among which the calcium-dependent cadherins represent a major multigene family of glycoproteins showing cell type-specific expression patterns (for reviews see Cunningham and Edelman, 1990; Kemler *et al.*, 1990; Takeichi, 1990, 1991). In some cases these cadherins are spread over large parts of the cell surface, whereas in other situations they show enrichment in intercellular junctions. For example, E-cadherin (uvomorulin) is accumulated in the adhering junctions of the *zonula adhaerens* of polar epithelial cells (Boller *et al.*, 1985), and N-cadherin («A-CAM») is enriched in the *fasciae adhaerentes* of the myocardium, in the extended intercellular adhering junctions of lens tissue and various cultured cell lines (Volk and Geiger, 1984, 1986) and in the special *zonulae adhaerentes* of certain epithelia during formation or a change of differentiation character (Geiger *et al.*, 1985, 1990).

A junctional specialization typical of epithelial differentiation but also occurring in myocardial, meningeal and also certain reticular and glial cells is the desmosome *(macula adhaerens)*, a mostly isodiametric membrane domain associated with a dense submembranous plaque. This plasma membrane specialization contains specific cytoplasmic plaque proteins, most prominently desmoplakin(s) and plakoglobin, and the transmembrane glycoproteins desmoglein and desmocollin (for reviews see Cowin *et al.*, 1985; Steinberg *et al.*, 1987; Garrod *et al.*, 1990; Green and Jones, 1990; Schwarz *et al.*, 1990). Determinations of amino acid sequences have led to the conclusion that both these desmosomal glycoproteins are members of the larger multigene family of cadherins (Koch *et al.*, 1990; see also Goodwin *et al.*, 1990; Holton *et al.*, 1990; Schwarz *et al.*, 1990), and this has since been confirmed by extensive cDNA analyses for several human and bovine desmogleins and desmocollins (Collins *et al.*, 1991; Koch *et al.*, 1991a,b; 1992; Mechanic *et al.*, 1991; Nilles *et al.*, 1991; Parker *et al.*, 1991; Wheeler *et al.*, 1991). In addition, it has been shown that each of these glycoproteins, desmoglein and desmocollin, again consists of a

Abbreviations used in this paper: bp, base pair(s); CAM, cell adhesion molecule; IF, intermediate-sized filaments; kb, kilobase(s); PCR, polymerase chain reaction; SDS PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.

***Address for reprints**: Division of Cell Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany. FAX: 49-6221-402598.

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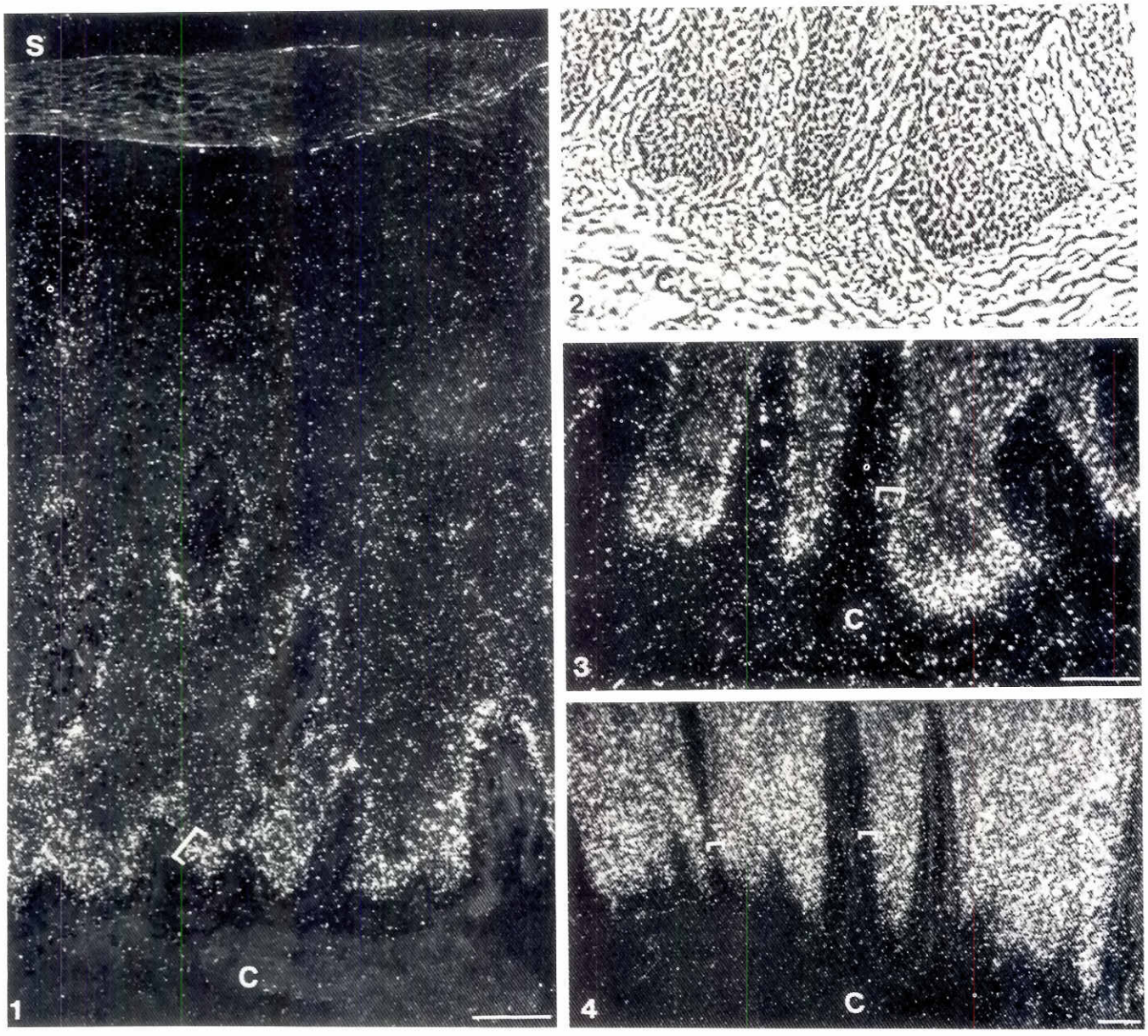


Figs. 1-4. Micrographs showing results of *in situ* hybridization experiments on frozen sections through bovine muzzle epithelium *(S, surface; C, connective tissue), using bovine cRNA probes specific for either type 2 desmocollin (dark field micrographs in Figs.* ***1*** *and* ***3****; Fig.* ***2****, phase contrast micrograph of the section in Fig.* ***3****) or type 1 desmocollin (Fig.* ***4****). Note that type 2 desmocollin is detected only in basal cell layers whereas desmocollin type 1 is expressed in suprabasal layers (Fig.* ***4****). The brackets denote the basal cell layer in the specific pictures. Bars, 100 μm.*

subgroup of related but distinct gene products that are expressed in different patterns in various kinds of desmosome-forming cells (Koch *et al.*, 1991a,b, 1992; for review see Buxton and Magee, 1992). Retrospectively, this observation of cell type-related diversity now seems to explain a series of earlier observations such as:

(i) cell type-related differences in immunoblot and immunohistochemical staining reactions (cf. Giudice *et al.*, 1984; Parrish *et al.*, 1986; Jones *et al.*, 1987; Schwarz *et al.*, 1990; Holton and Garrod, 1992), which also might have been caused by selective epitope masking;

(ii) amino acid exchanges of short proteolytic fragments (King *et al.*, 1991) which could as well reflect allelic differences; and

(iii) differences in SDS-PAGE mobility (Cohen *et al.*, 1983; Suhrbier and Garrod, 1986; Kapprell *et al.*, 1990) which alternatively might result from different kinds or degrees of protein modification or of proteolysis.

Historically, bovine tissues have been mostly used in biochemical research on desmosomal components, and in particular the bovine muzzle epithelium is the best studied source of isolated desmosomes (cf. Skerrow and Matoltsy, 1974a,b; Drochmans *et*

al., 1978; Franke *et al.*, 1981; Gorbsky and Steinberg, 1981; Mueller and Franke, 1983). To allow studies of the expression of different desmocollin genes in human tissues, we have therefore isolated cDNAs encoding different human desmocollins and examined the distribution of desmocollin mRNAs in human tissues.

Results

In the course of our studies on the distribution of the two types of bovine desmocollins, then termed BMDCT1 and BMDCT2, we noted differences in mRNA synthesis and concentration in various bovine stratified epithelia, including muzzle epithelium (Koch *et al.*, 1992). Using *in situ* hybridization, we found that the probe representing bovine type 2 desmocollin (BMDCT2) showed intense labeling of all living cell layers of, e.g., bovine tongue mucosa (cf. Fig. 6 of Koch *et al.*, 1992) but not in muzzle epithelium where it was clearly enriched in the basal cell layers and practically negative in the uppermost strata (Figs. 1-3). At higher resolution it became evident that the basalmost cell layer, which was positive for this type of desmocollin mRNA (Figs. 2 and 3), was only weakly — or not at all — labeled with the desmocollin type 1 (BMDCT1) probe, which in turn reacted very intensely with the suprabasal cell layers (Fig. 4).

To examine the cell layer distribution of these two types of desmocollins in human tissues, normal or malignant, we decided to isolate cDNA clones encoding mRNAs for the corresponding two types of human desmocollins. To this end we probed human epidermal cDNAs with bovine cDNA probes of both desmocollins in their large splice variant, termed «*a*» or «*I*» (Fig. 5; cf. Koch *et al.*, 1991b, 1992).

Isolation and characterization of a cDNA clone encoding human desmocollin type 1

Using the bovine type 1 desmocollin cDNA, clone BMDCT1-BDC7-5, we isolated a phage λ-encoded clone of 4194 bp (HEDCT1-9) from a human foreskin epidermal cDNA expression library. Due to an internal EcoRI restriction site this clone yielded two subclones, HEDCT1-9.2 and 9.4. Clone 9.4 Pst, which in hybridization experiments reacted with a ~6.4 kb mRNA present in human breast epidermis (data not shown here), presented an open reading frame corresponding to a desmocollin precursor polypeptide of more than 903 amino acids, followed by a 3'-untranslated region of 1485 nucleotides, including another reading frame of 65 aminoacids and a 16 residues-long oligo A-stretch which, however, most probably was not a residue of the polyadenylation region (Fig. 6). This clone, while comprising the entire mature, processed proteins of both splice forms *a (II)* and *b (II)*, is not a complete cDNA of desmocollin mRNA as it contains neither the start codon and the 5'-untranslated sequence, nor the very 3'-end of the mRNA and its poly(A)-tail.

Fig. 6 presents the nucleotide sequence of the cDNA and the deduced amino acid sequence of the precursor protein, as far as it is encoded in the clone. The site of proteolytic processing, resulting in the amino-terminus of the mature protein (indicated by arrows in Figs. 6 and 7), is readily identified by its homology to the amino-terminus of the bovine protein, the amino acid sequence of which has previously been determined directly (cf. Holton *et al.*, 1990; Fig. 2 of Koch *et al.*, 1991b).

Fig. 7 shows both the homology and the difference between the type 1 desmocollin encoded by this clone (HDCT1) and the type 2 desmocollin (HDCT2) as reported by Parker *et al.* (1991). It is obvious from this comparison that short segments showing sequence identity or similarity are separated by regions diversified in sequence or by short deletions or insertions. Such clusters of homology occur in the extracellular as well as in the cytoplasmic portion and very significantly also extend into the precursor segment that is lost in the mature protein. Particularly high is the homology at the carboxy-terminus (Fig. 7 shows the splice variant *b* form, the last 11 aminoacids of which are encoded by the «mini-exon»; cf. Collins *et al.*, 1991; Mechanic *et al.*, 1991; Parker *et al.*, 1991; Troyanovsky *et al.*, 1993).

On the other hand, comparison with the bovine desmocollin sequences published (cf. Koch *et al.*, 1991b, 1992) reveals the

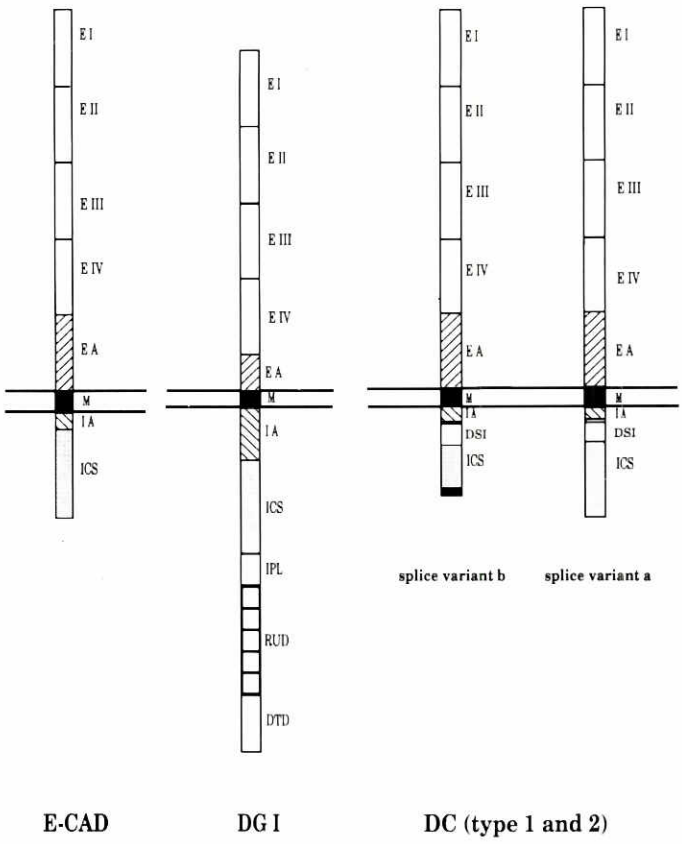


Fig. 5. Schematic presentation of the molecular structure and topological organization of different cadherins. *The individual domains of E-cadherin (E-CAD), epidermal desmoglein I (DG I) and the two splice variants (a and b or I and II) typical of desmocollins (DC; the example shown here represents type 1 desmocollin) are indicated. Note that the two desmocollin splice variants differ only in their carboxy-terminal, cytoplasmic domain. The black box at the carboxy-terminus of splice variant b indicates the 11 amino acids encoded by the «mini-exon» (cf. Franke et al., 1992). The individual domains are designated as follows: EI - EIV, extracellular repeating elements; EA, extracellular anchoring domain; M, transmembrane domain; IA intracellular anchoring domain; DSI, desmocollin-specific insertion; ICS, intracellular cadherin-typical sequence; IPL, intracellular proline-rich linker; RUD, domain containing five repeating elements; DTD, desmoglein-specific terminal domain. For detailed descriptions of these domains see Koch et al. (1990, 1991a,b, 1992) and Troyanovsky et al. (1993).*

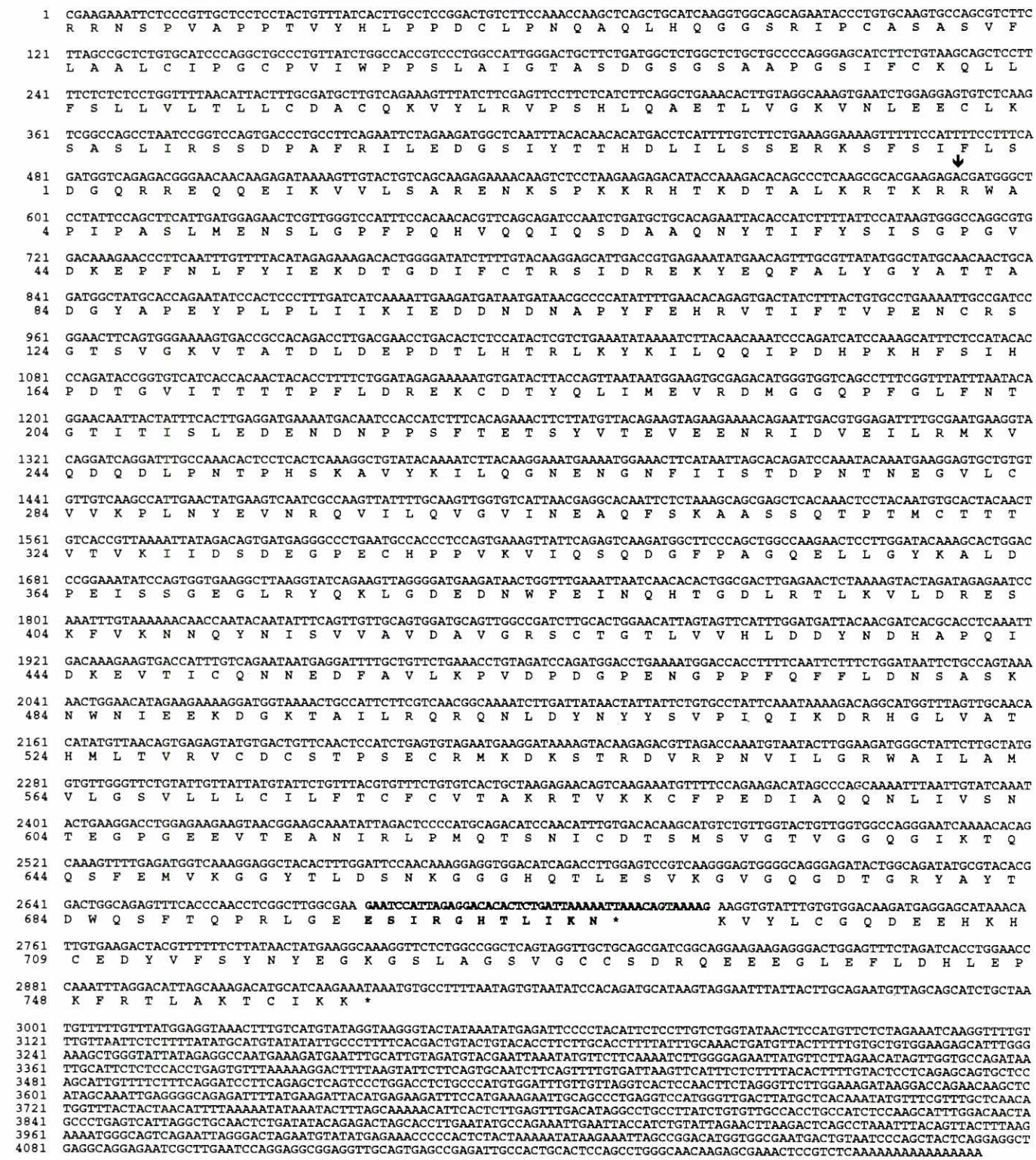


Fig. 6. Nucleotide sequence and deduced amino acid sequence (one-letter-code) of cDNA encoding human type 1 desmocollin. *The sequence of clone HEDCT1-9 encodes the entire mature protein and most of the precursor-specific portion but does not include the amino-terminus of the complete precursor and the complete 5'-untranslated part of the mRNA. The arrow indicates the proteolytic cleavage site for the generation of the mature polypeptide. The stop codon of splice variant b, derived from the «mini-exon» printed in bold face letters, is designated by the first asterisk. The second asterisk denotes the stop codon of splice variant a which starts at position 2722, resulting in the new splice transition RLGE/KVYL and ends with CIKK. The 3'-end oligo-A-stretch is most probably not the start of the polyadenylation region as it is not preceded by a typical polyadenylation signal (cf. Birnstiel et al., 1985).*

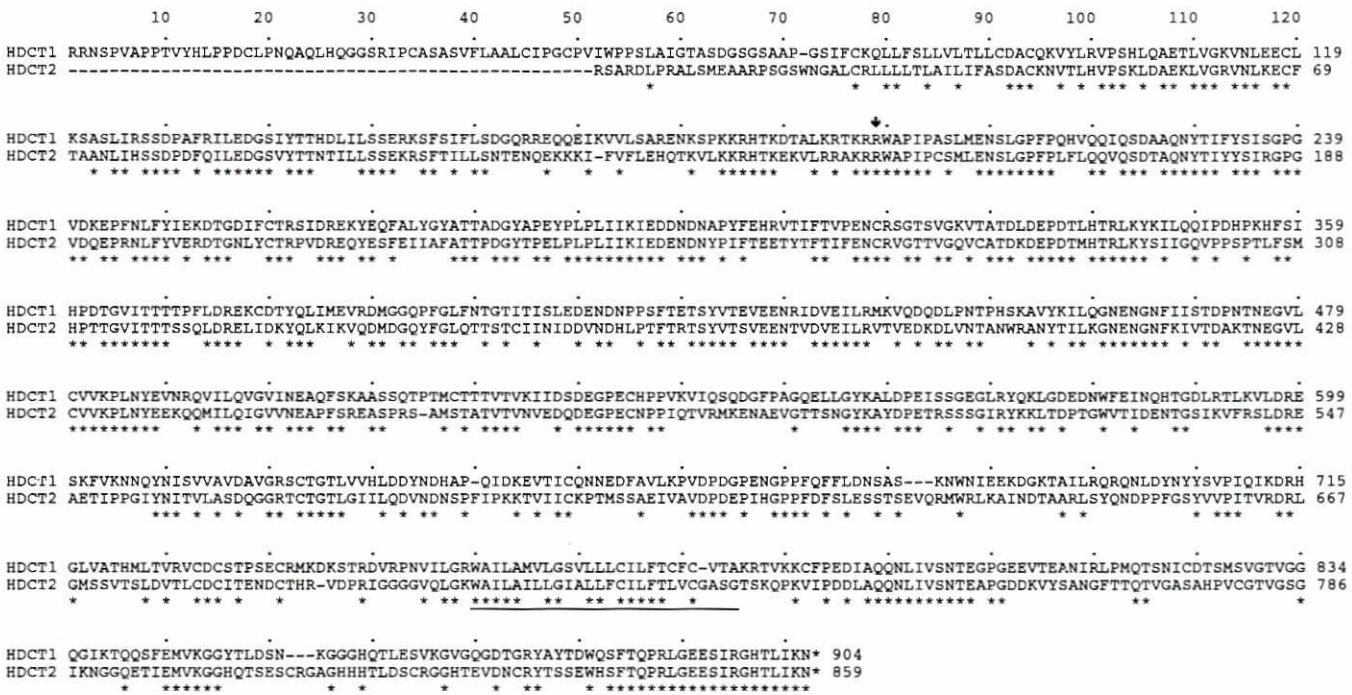


Fig. 7. Amino acid sequence comparison of the human type 1 desmocollin (HDCT1) with the human type 2 desmocollin (HDCT2). *Both sequences (only splice is shown) do not include the complete mRNA and the amino-terminus of the precursor polypeptide (the HDCT2 sequence taken from Parker et al., 1991). The cleavage site resulting in the formation of the amino-terminus of the mature polypeptide is indicated by the arrow, the transmembrane portion is underlined. Identical amino acids are indicated by asterisks and certain conservative exchanges by dots (not considered here are exchanges of the aromatic residues Y and F and the hydrophobic nature of F). Note considerable sequence differences between these two human desmocollins.*

sequences published (cf. Koch *et al.*, 1991b, 1992) reveals the corresponding desmocollin types in the two species, as demonstrated in Fig. 8. The high degree of sequence homology (81% identical and 89% homologous amino acid residues in splice variant *b*) strongly suggests that the human desmocollin presented here (HDCT1) is the interspecies desmocollin type 1 counterpart of the bovine protein BMDCT1, i.e. derived from an orthologous gene.

The total molecular weight of the mature polypeptide chain of 706 amino acids (splice variant *b*) can be calculated as 78,815 which is very similar to the value of 79,044 determined for the 707 amino acids of splice variant *b* of bovine type 1 desmocollin (cf. Koch *et al.*, 1991b). The longer splice variant *a* (Fig. 6) comprises 760 amino acids, corresponding to a molecular weight of 85,000. The previous higher molecular weight estimates based on SDS-PAGE (for refs. see Introduction) are — at least partly — due to glycosylation (cf. Gorbsky and Steinberg, 1981; see also Kapprell *et al.*, 1985, 1990).

Isolation and characterization of a cDNA clone encoding human desmocollin type 2

Using the PCR technique we isolated a human cDNA (HEDCT2-15) corresponding to the desmocollin described by Parker *et al.* (1991), as described in Materials and Methods. The segment of the polypeptide corresponding to the 839 nucleotides of this clone includes the membrane-spanning segment as well as 158 amino acids of the extracellular and 98 amino acids of the cytoplasmic portion.

Expression of genes encoding type 1 and type 2 desmocollins in stratified epithelia as visualized by in situ hybridization

When frozen samples of human epidermal tissue from various sources were examined by *in situ* hybridization, using probes specific either for desmocollin type 1 (subclone HEDCT1-9.2 *Pst*1) or desmocollin type 2 (subclone HEDCT1-15), most living cell layers were intensely labeled (Figs. 9 and 10). Closer inspection, however, revealed that the basal cell layer was not significantly labeled with the HEDCT1 probe (Fig. 9a, inset) but clearly positive for type 2 desmocollin (Fig. 10a). The epidermal tissue surrounding the pilosebaceous tract was also strongly positive for both desmocollins (Figs. 9 and 10), whereas the associated glandular epithelium appeared weakly positive for desmocollin type 2 (Fig. 10a) but negative for type 1 desmocollin.

Several other stratified epithelial tissues tested were also rich in type 2 desmocollin mRNA. Examples include the exocervical epithelium (Fig. 11a,b) and the esophageal mucosa (Fig. 12a-d) which were both strongly labeled with the type 2-specific probe. In both tissues, however, the labeling was clearly restricted to the basal part of the mucosa, whereas the upper strata showed only weak — if any — label. In contrast, we did not observe significant labeling with the desmocollin type 1-specific probes (data not shown).

Discussion

The molecule characterized in this study by the nucleotide sequence of its mRNA is undoubtedly the human ortholog of the

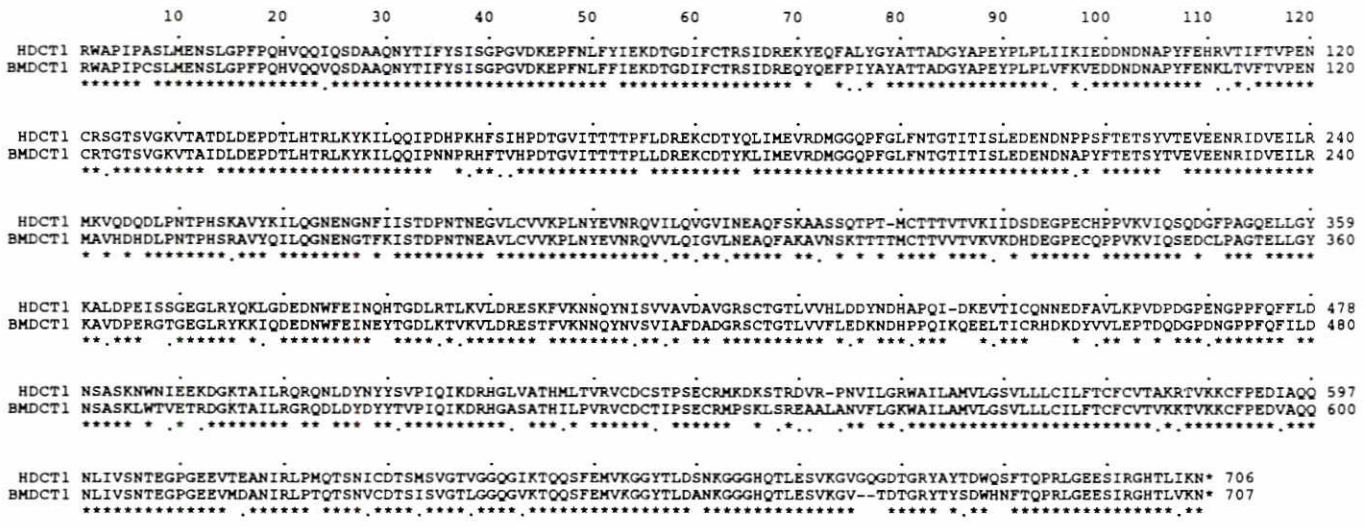


Fig. 8. Amino acid sequence comparison of human type 1 desmocollin with the corresponding bovine protein *(for symbols see Fig. 7). The high degree of identical amino acids (asterisks) indicates that these polypeptides (only splice variant b is shown) are encoded by orthologous genes in the two species.*

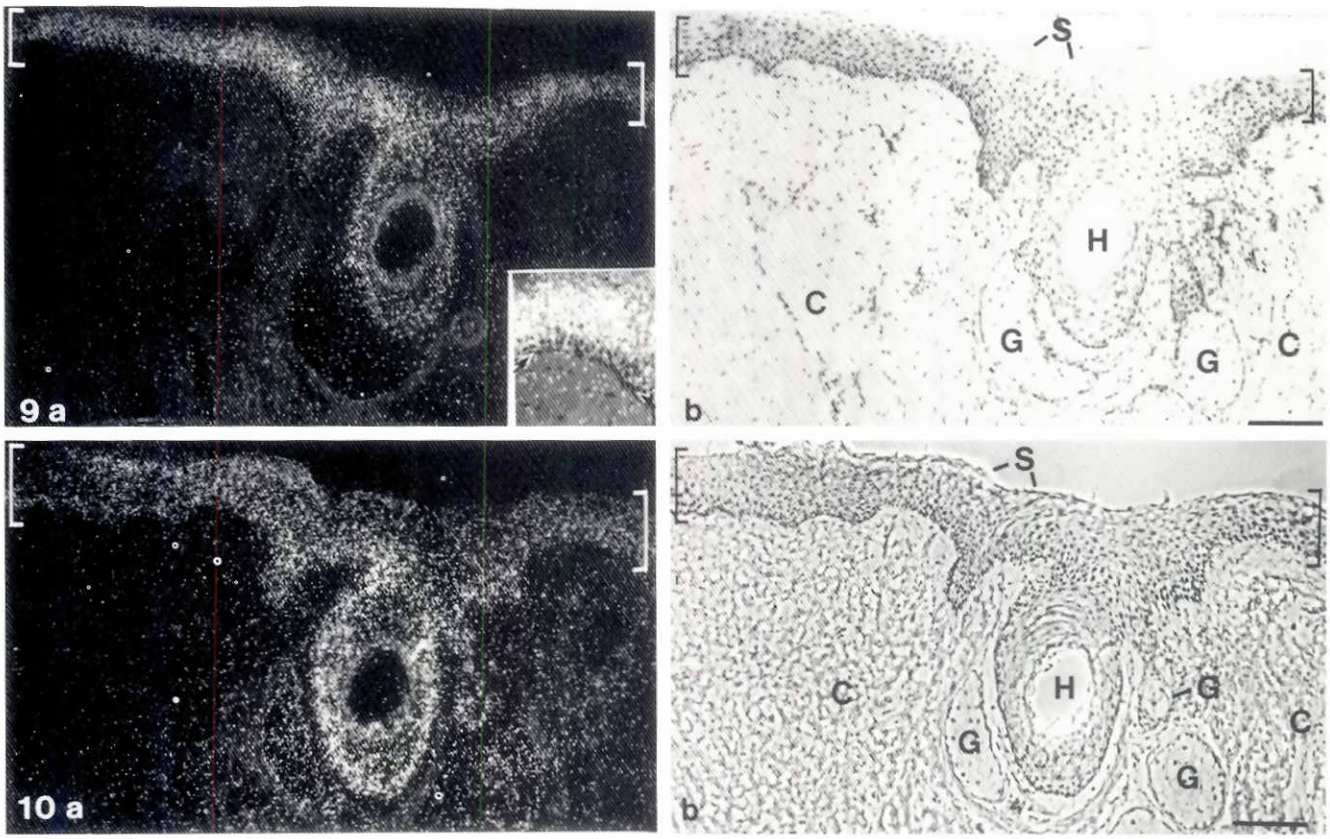
bovine type 1 desmocollin presented by us (Koch *et al.*, 1991b) and others (Collins *et al.*, 1991; Mechanic *et al.*, 1991) and is clearly different from the type 2 desmocollin described in human (Parker *et al.*, 1991) as well as bovine (Koch *et al.*, 1992) tissues. The differences between the amino acid sequences of these two types of desmocollins in the same species are remarkably high, including the cytoplasmic («tail») domain recently shown to be of functional importance in the formation of a desmosomal plaque and IF anchorage (Franke *et al.*, 1992; Troyanovsky *et al.*, 1993).

This tail domain is 14 amino acids shorter in the type 1 desmocollin and, in the shorter form of splice variant *b* (124 amino acids), contains only three, relatively small, «islands» of sequence conservation between the two types of human desmocollins compared in Fig. 7: one extending from residue 6 (lysine) after the membrane-spanning region to residue 28, a short segment between residues 61 and 70, and the 26 carboxy-terminal residues. The functional meaning of these sequence differences between two types of human desmocollins — or between the three different desmocollin genes already identified in the bovine genome (cf. Troyanovsky *et al.*, 1993) — is not clear and is currently being tested in our laboratory by cell transfection experiments, using deletions and point mutations introduced into cDNA clones.

It is also evident from the results of this and a previous study (Koch *et al.*, 1992) that in both species, cow and man, the type 2 desmocollin is much more widespread than the type 1 protein. While we have identified mRNA encoding type 2 desmocollin in all of the various stratified epithelia examined as well as in simple epithelia, myocardium and lymph nodes (P.J. Koch and W.W. Franke, unpublished results), type 1 desmocollin was detected by *in situ* hybridization only in human and bovine epidermis, in the special tissue of the bovine muzzle epithelium and also — in very low amounts — in bovine tongue mucosa. At present we cannot decide whether the negative results in so many desmosome-forming tissues are due to the absence of type 1 desmocollin mRNA or to an extremely low concentration.

Our *in situ* hybridization results showing, in both species, type 2 desmocollin mRNA in several basal layers, including the basalmost one, but desmocollin type 1 mRNA only in suprabasal cell layers, indicate a further restriction of type 1 desmocollin mRNA synthesis and accumulation, probably also of the expression of the gene. This restriction of synthesis to suprabasal cell layers in epidermis and a few related stratified epithelia is reminiscent of the pattern of synthesis reported for certain IF proteins such as epidermal cytokeratins 1, 2 and 10, i.e. components of the IFs anchoring at desmosomes containing type 1 desmocollin (e.g. Fuchs and Green, 1980; Woodcock-Mitchell *et al.*, 1982; Jorcano *et al.*, 1984; Fuchs *et al.*, 1987; Kopan *et al.*, 1987; O'Guin *et al.*, 1987; Stoler *et al.*, 1988; Collin *et al.*, 1992a,b). Thus, our present study adds type 1 desmocollin to the list of molecules which are synthesized in relation to suprabasal and terminal differentiation in certain stratified epithelia. From these findings one might also suggest that desmocollin type 1 is functionally involved in the suprabasal differentiation and in the cell-cell adherence of terminally differentiating keratinocytes. Whether specific type 1 desmocollin features also contribute to the known altered desmosomal structure in the uppermost layers, i.e. those of the *stratum granulosum* and *stratum corneum* (for refs. see Montagna and Parakkal, 1974), and to the desquamation of cell remnants at the epidermal surface remains to be studied.

Antibodies against desmosomal constituents have been successfully used for immunocytochemical cell typing in tumor diagnosis, most importantly for the detection and the classification of carcinoma cells (e.g., Franke *et al.*, 1983; Moll *et al.*, 1986; Parrish *et al.*, 1986, 1987; Schmelz *et al.*, 1986a,b; Vilela *et al.*, 1987). The discovery that desmogleins and desmocollins exist in different isoforms which can either coexist or are differentially synthesized (this study and Koch *et al.*, 1991a,b, 1992) now opens the possibility to use antibodies specific for the individual isoforms in tumor diagnosis, notably in the characterization of squamous cell carcinomas and their metastases.



Figs. 9 and 10. *In situ* hybridization showing the expression of type 1 and type 2 desmocollins in human epidermis. *Micrographs showing the silver grain distribution on frozen sections through human epidermis (in this case from forehead skin) after in situ hybridization, using ^{35}S-labeled cRNA probes specific for type 1 (Figs.* ***9a,b****; HEDCT1-9.2 Pst1) or type 2 (Figs.* ***10a,b****; HEDCT2-15) desmocollin. The silver grains (exposure for 10 days) are seen in dark field illumination (Figs.* ***9a*** *and* ***10a****), whereas the corresponding tissue structures are shown in bright field (Fig.* ***9b****) or phase contrast optics (Fig.* ***10b****). S, epidermal surface (note that most of the stratum corneum has been lost in the samples shown here); C, connective tissue of dermis; H, hair follicle; G, glandular epithelium of sebaceous glands. Note intense labeling with both probes on suprabasal living cell layers of epidermis (denoted by brackets) but not in the stratum corneum residues detectable in the upper left of Figs.* ***10a*** *and* ***10b****. The basal cell layer is practically negative with the type 1 desmocollin probe (resolved in the partial higher magnification shown in the inset of Fig.* ***9a****) but positive for type 2 desmocollin. Bars, 100 μm.*

Materials and Methods

Tissues

Samples of human epidermis from various body sites, including breast and forehead skin and of other tissues (e.g. tongue, esophagus, exocervix) were obtained after surgery for various medical reasons or during autopsy and immediately frozen in isopentane, cooled with liquid nitrogen to -130°C and stored at -70°C (Collin *et al.*, 1992a,b). Bovine epithelial tissues were excised, frozen and used as described (Franke *et al.*, 1981; Bosch *et al.*, 1988; Koch *et al.*, 1992).

Screening, cloning and sequencing of cDNA

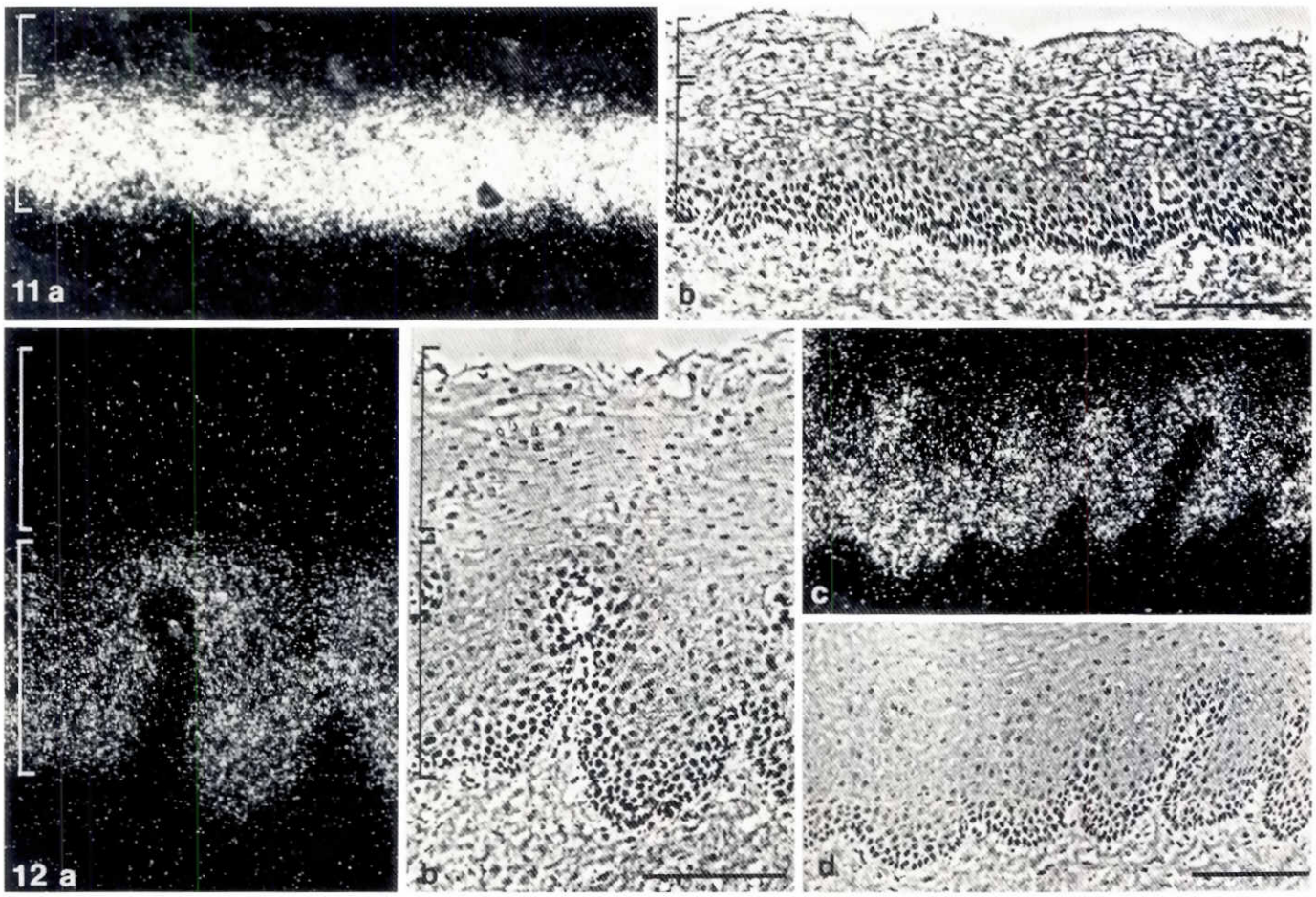
A human foreskin λgt11 expression library (Clontech, Heidelberg, FRG) was screened with a ^{32}P-labeled cDNA probe of subclone BDC7-5 of BMDCT1, encoding the bovine muzzle epithelial Type 1 desmocollin, form a (or *I*; cf. Koch *et al.*, 1991). Positive phages were plaque-purified three times. Upon restriction digestion of the purified clone HEDCT1I-9 with EcoRI two subclones were obtained, HEDCT1I-9.2 and HEDCT1I-9.4, each of which was subcloned in Bluescript (Stratagene, Heidelberg, FRG) or M13 BM20RF and M13 BM21RF (Boehringer, Mannheim, FRG) vectors. Both strands of both subclones were sequenced using the T7 sequencing kit (Pharmacia, Freiburg i. Br., FRG).

Polymerase chain reaction (PCR)

A partial cDNA clone of the human type 2 desmocollin (Parker *et al.*, 1991) was generated by PCR as described in detail elsewhere (cf. Collin *et al.*, 1992a,b).

Briefly, 10 μg of total RNA from human breast epidermis were heated at 65°C for 3 min, chilled on ice, and reverse-transcribed in 20 μl of »reverse transcription buffer« (50 mM Tris-HCl, pH 8.15 at 41°C, 6 mM $MgCl_2$, 40 mM KCl, 1 mM DDT, each dNTP at 1.5 mM) containing 20 units of »RNasin« (Pharmacia), 0.74 μg of a random mixture of hexanucleotides (Pharmacia) and 20 units of avian myeloblastosis virus reverse transcriptase (Boehringer). The reaction mixture was incubated for 1 h at 42°C, 30 min et 52°C, denatured 5 min at 95°C and diluted to 1 ml with »TE buffer« (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA). 5 μl aliquots of the first cDNA strand were subjected to amplification by PCR, using a set of two specific primers: a synthetic oligonucleotide 5'-GGCAAGCCTGCAGAGACCATC-3' (positions 1649-1660) was applied in combination with a 3'-end oligonucleotide 5'-GGCGGATCCCACCTCCGTGTGTCC-3' (complementary to positions 247 - 2488 of the human protein; cf. Parker *et al.*, 1991).

Amplification was performed in 100 μl of »PCR buffer« (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 0.01% BSA) containing 200 μM of each dNTP, 25 pM of both primers and 2.5 units Ampli-Taq (Perkin-Elmer Cetus, Norwalk, CT, USA). Forty cycles of amplification (denaturation, 0.5 min at



Figs. 11 and 12. Synthesis of mRNA encoding type 2 desmocollin in human exocervix (Fig. 11) and esophagus (Fig. 12) as revealed by *in situ* hybridization. *Intense silver grain labeling (exposure time: 3 days) of the lower strata of both mucosae (frozen sections) is seen in dark field illumination (Figs.* ***11a****,* ***12a*** *and* ***12c****; the corresponding phase contrast images are shown in Figs.* ***11b****,* ***12b*** *and* ***12d****), whereas the upper strata are not significantly labeled (the two regions are demarcated by the brackets on the left margin of Figs.* ***11a*** *and* ***12a****). The restriction of mRNA synthesis to the basal cell layers is particularly evident from the oblique section shown in Figs.* ***12c*** *and* ***12d****. Bars, 100 μm.*

94°C; annealing, 1 min at 60°C; extension, 2 min at 72°C) were followed by 10 min elongation at 72°C. The PCR product was purified and cloned in Bluescript vector (Stratagene) and termed HEDCT2-15.

In situ hybridization

The procedure used for *in situ* hybridization on sections of frozen tissue samples was as described (Bosch *et al.*, 1988; Collin *et al.*, 1992a,b). Antisense cRNA probes of human type 1 (positions 840-2831; obtained by PstI digestion of clone HEDCT1-9) and type 2 (HEDCT2-15) desmocollins were synthesized using [α-[^{35}S]thio]CTP (Amersham-Buchler, Braunschweig, FRG) according to standard procedures. For studies of bovine tissues, probes corresponding to position 1695-1406 of bovine type *1* desmocollin (Koch *et al.*, 1991b) and position 1952-1491 of bovine type 2 desmocollin (Koch *et al.*, 1992) were used.

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