

The Complement of Desmosomal Plaque Proteins in Different Cell Types

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ABSTRACT Desmosomal plaque proteins have been identified in immunoblotting and immunolocalization experiments on a wide range of cell types from several species, using a panel of monoclonal murine antibodies to desmoplakins I and II and a guinea pig antiserum to desmosomal band 5 protein. Specifically, we have taken advantage of the fact that certain antibodies react with both desmoplakins I and II, whereas others react only with desmoplakin I, indicating that desmoplakin I contains unique regions not present on the closely related desmoplakin II. While some of these antibodies recognize epitopes conserved between chick and man, others display a narrow species specificity. The results show that proteins whose size, charge, and biochemical behavior are very similar to those of desmoplakin I and band 5 protein of cow snout epidermis are present in all desmosomes examined. These include examples of simple and pseudostratified epithelia and myocardial tissue, in addition to those of stratified epithelia. In contrast, in immunoblotting experiments, we have detected desmoplakin II only among cells of stratified and pseudostratified epithelial tissues. This suggests that the desmosomal plaque structure varies in its complement of polypeptides in a cell-type specific manner. We conclude that the obligatory desmosomal plaque proteins, desmoplakin I and band 5 protein, are expressed in a coordinate fashion but independently from other differentiation programs of expression such as those specific for either epithelial or cardiac cells.

Desmosomes are domains of the plasma membrane that are structurally specialized, on their cytoplasmic face, to form membrane anchorage sites for intermediate filaments and, on their intercellular face, to mediate strong cell-cell adhesion (for reviews, see references 1–3). The architectural coincidence of these two interactions makes the desmosome-tonofilament complex ideally suited to impart tensile strength and mechanical resistance to the tissue as a whole.

Desmosomes are most abundant in stratified epithelial tissues, and our current knowledge of their biochemical composition is based on analyses of preparations enriched in desmosomes from these sources (4–11). Preparations of desmosomes from bovine snout epidermis can be resolved on gel electrophoresis into eight major polypeptide bands, and at least six individual proteins have been identified as true desmosomal constituents. These are the nonglycosylated proteins, desmoplakins I ($M_r \sim 250,000$), desmoplakin II ($M_r \sim 215,000$), and polypeptide “band 5” ($M_r \sim 83,000$), as well as the glycosylated proteins of bands 3 ($M_r \sim 150,000$), 4a (M_r

$\sim 130,000$), and 4b ($M_r \sim 115,000$). In addition, a basic polypeptide band 6 ($M_r \sim 75,000$) co-purifies in desmosomal preparations, often together with minor amounts of a smaller glycosylated component with an M_r of approximately 22,000.

Desmosomes also occur, albeit at much lower frequency, in other epithelia, i.e., pseudostratified, transitional, and simple epithelia (for reviews, see references 1, 12, and 13). In addition, they have been found in certain arachnoidal cells of the meninges, in meningiomas derived therefrom (14) and within the intercalated discs of the myocardium, a tissue of mesenchymal origin (15, 16). Antibodies to desmoplakins I and II (10, 17, 18) and to several other desmosomal components (19–22) have been shown, using fluorescence microscopy, to react at desmosomal sites in various cell types in a wide variety of species. However, the biochemical composition of desmosomes from sources other than epidermis and a few other stratified epithelia has not been studied due to the difficulty in detecting such minor cellular components. Clearly, it is important to establish whether desmosomes from

different sources are identical in their composition or whether they constitute, as is the case with many other cytoskeletal proteins, structures composed of members of a family of proteins showing differential expression in different cell types.

In the present study, we have addressed this question with regard to the intracellular components desmoplakins I and II and band 5 protein.¹ Immunoelectron microscopy using conventional antisera has shown these polypeptides to be associated with the desmosomal plaques (8, 10, 17, 18). Desmoplakins I and II are very similar to each other in their range of isoelectric pH values (~6.5–7.2), amino acid composition, and peptide maps (10, 11) but differ in all of these features from band 5 protein (10, 11, 23). Immunologically related polypeptides of similar sizes to desmoplakins I and band 5 protein have been described in certain bovine stratified tissues (10, 24), but a systematic investigation of the desmosomal plaque protein complement in the diverse kinds of desmosome-containing cell types is still lacking.

We have produced a panel of monoclonal antibodies to the desmoplakins and a guinea pig antiserum to band 5 protein and used these to identify, by immunoblotting, the cross-reacting proteins in a number of cultured cells and tissues from several species. These include, in addition to various stratified epithelia, examples of simple, transitional, and pseudostratified epithelia as well as cardiac tissue. The results show that the desmosomal plaque complement of proteins is relatively uniform in all cell types. However, while desmoplakins I and band 5 protein are present in all desmosomes, desmoplakins II has only been found among cells of stratified epithelial tissue.

MATERIALS AND METHODS

Antibodies: The monoclonal antibodies described were the result of two fusions. In the first, the antigen comprised preparations of isolated bovine snout desmosomes, and in the second, desmoplakins purified from such preparations by elution from SDS PAGE (22). Two injections of 500 µg of antigen emulsified 1:1 initially with Freund's complete adjuvant and then with Freund's incomplete adjuvant were administered, subcutaneously, with a 4-wk interval, to BALB/c mice. 3 d before fusion, 500 µg of antigen suspended in PBS was injected intraperitoneally. Spleen cells were fused with a myeloma cell line NSO in a ratio of 5:1, respectively, using 40% polyethylene glycol 4,000 (25). After fusion, cells were distributed into 10 flat-bottomed microtiter plates (24 wells each, Costar, Technomara, Fernwald, FRG) containing a feeder layer of spleen cells at a concentration of 5×10^4 /well. The cells were cultured in HAT (hypoxanthine, aminopterin, thymidine)-RPMI medium supplemented with 10% fetal calf serum (Flow Labs, Irvine, UK), 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1 mM sodium pyruvate. Screening for antibody activity in the culture supernatants was carried out by immunofluorescence microscopy on frozen sections of cow snout and by immunoblotting of bovine snout desmosomal proteins. Positive hybridomas were subcloned twice by limiting dilution in 96-well microtiter plates. After subcloning, hybridomas were weaned off HAT-RPMI into RPMI and grown in 150-cm² flasks or propagated as ascites in pristane-treated BALB/c mice.

Guinea pigs were given three injections of individual desmosomal polypeptides purified by SDS PAGE. Protein was emulsified with complete Freund's adjuvant (500 µg/injection) for the first and incomplete Freund's adjuvant for subsequent injections that were administered subcutaneously and intramuscularly with 4–6 wk intervals between each.

¹ Steinberg and colleagues (8) have proposed the name desmoplakins III for the band 5 protein, which implies a close chemical relationship to desmoplakins I and II, similar to that of members of other protein families such as collagens, keratins, and others. However, as band 5 protein is very different in its immunological and biochemical properties from desmoplakins I and II, we think this name is misleading and prefer to refrain from using it unless sequence comparison reveals significant homologies.

Fluorescence Microscopy: Bovine tissues were obtained from the local slaughterhouse, and human biopsy specimens and tissues of rat (Sprague-Dawley) and White Leghorn chicken were snap-frozen in liquid nitrogen-cooled isopentane. 5-µm sections were prepared with a Reichert cryotome (Reichert Scientific Instruments, Buffalo, NY) and air-dried. Fixation for 10 min in acetone (–20°C) was followed by brief air-drying. Primary antibodies were applied for 30 min, followed by three 5-min washes in phosphate-buffered saline (PBS), 30 min of incubation with fluorescein isothiocyanate-coupled goat anti-mouse or guinea pig Ig (Medac, Hamburg, FRG) diluted 1:20, and three washes (5 min each) in PBS as before. The slides were dipped briefly in water, then in ethanol, and coverslips were mounted in Moviol (Hoechst, Frankfurt, FRG). The sections were viewed using epifluorescence illumination, with a Zeiss photomicroscope III (Carl Zeiss, Inc., Oberkochen, FRG).

Immunoelectron Microscopy: Sections were fixed and stained as described (14).

Gel Electrophoresis: SDS PAGE was as described (26). For two-dimensional gel electrophoresis the method of O'Farrell et al. (27) with some modifications (10, 28) was used.

Immunoblotting: Polypeptides separated by electrophoresis were incubated for 2 h in partial renaturing buffer as described (29). They were then transferred to nitrocellulose paper (30) and the efficiency of the procedure checked by staining with Ponceau S (Sigma Chemical Co., St. Louis, MO). Nonspecific binding was reduced by incubation with 0.05% Tween in PBS for 2 h. The nitrocellulose paper was reacted with hybridoma culture supernatant or with IgG (0.003 mg/ml) purified from ascites fluid or with guinea pig antiserum diluted 1:500. Four 20-min washes were given with wash buffer (PBS, 0.05% Tween) containing sequentially 0.1% Triton X-100, 0.5% Triton X-100, and 0.5 M NaCl, and finally wash buffer alone. Affinity purified ¹²⁵I-labeled goat antibodies to mouse IgG + IgM (5×10^4 cpm/ml) or ¹²⁵I-labeled protein A (Amersham International, Amersham, UK) was applied for 2 h, and then the paper was washed again as described above.

Alternatively, a peroxidase immunoblotting method was used as follows. First and second antibodies (rabbit anti-mouse IgG + IgM from Dakopatts, Denmark; diluted 1:1,000) were each incubated for 1 h. Washing steps comprised three 10-min washes in 0.05% Tween (in PBS). Specifically bound antibody was visualized by addition of diaminobenzidine (0.04%), nickel sulfate (0.05%), and peroxidase (0.03%) as substrate. The reaction was stopped by washing with PBS.

Enriched Desmosome Preparations: Desmosomes were prepared from bovine snout and tongue according to the method of Gorbisky and Steinberg (6). Desmosome-enriched fractions of bovine cornea, esophagus, bladder urothelium, trachea, and rat tongue were prepared as follows: The epithelial layers were scraped off and incubated in buffer A (96 mM NaCl, 8 mM KH₂PO₄, 5.6 mM Na₂PO₄, 1.5 mM KCl, 10 mM Na₂EDTA, pH 6.8), then homogenized in a Polytron homogenizer (Kinematica, Lucerne, Switzerland) for 1 min at setting 6. The homogenate was filtered and centrifuged for 1 min at 500 g to remove large debris. The supernatant was then further centrifuged at 2,000 g for 30 min and the resulting pellet taken up in buffer B (20 mM phosphate buffer, pH 7.5, containing 1.5 M KCl, 1% Triton X-100, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) and incubated for 45 min at 4°C with stirring. The homogenate was centrifuged at 10,000 g for 20 min, and the resulting pellet was washed twice in PBS, then stored as a pellet at –20°C.

Bovine and rat heart were minced with scissors, homogenized briefly, and then prepared according to the above procedure. Samples of human tongue epithelium and human epidermis (obtained as autopsy or biopsy samples, respectively) were microdissected and then prepared as above. Brush border cytoskeletal preparations from rat small intestine were prepared using dissociating buffer conditions as described (31). Bovine and rat liver cytoskeletal fractions were prepared as described (32).

Bovine mammary gland epithelial cells (lines BMGE + HM and BMGE – H) were cultured as described (33, 34). Cells were labeled with [³⁵S]methionine as described (33). Human cell lines, i.e., MCF-7 derived from a breast carcinoma (35), A-431 derived from a human adenocarcinoma of the vulva (36), and TR-146 cells derived from a metastatic squamous cell carcinoma of the cheek (37) were grown as described (38). Human keratinocytes were the kind gift of Dr. N. Fusenig (Department of Biochemistry, German Cancer Research Center) and were cultured as described (39). Cytoskeletal fractions resistant to high salt buffer and Triton X-100 were prepared according to Franke et al. (28).

RESULTS

Specificity of Monoclonal Antibodies

For the purpose of this study, we have selected seven different hybridomas (all IgG1) that produce desmoplakins

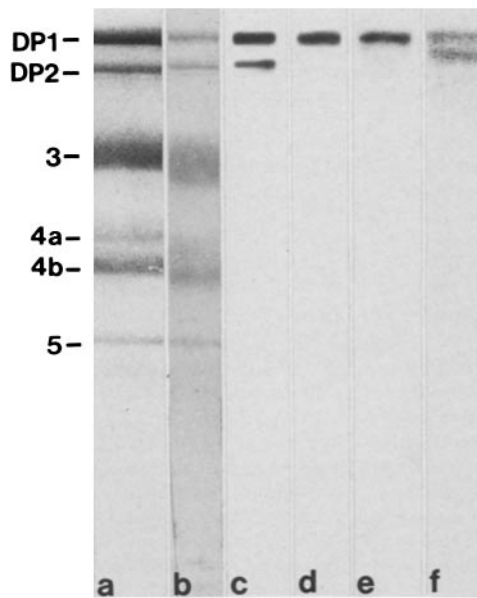


FIGURE 1 Specificity of desmoplakin antibodies demonstrated by immunoblotting reaction on polypeptides of desmosomes isolated from bovine snout epidermis after separation by SDS PAGE. Lane a, Coomassie Blue staining of major polypeptides of desmosomes

antibodies recognizing different epitopes. Representatives of the four major categories shown in Fig. 1 include (a) antibodies that react with both desmoplakins I and II of all species examined, (b) antibodies reacting only with desmoplakin I, (c) antibodies showing wide species specificity, and (d) antibodies showing species restriction (see Table I for details).

Positive and specific immunoblotting reaction for desmoplakins has been found in all stratified epithelia by both SDS PAGE (Fig. 1; see also below) and two-dimensional nonequilibrium pH gradient electrophoresis (for examples, see Fig. 2, a and b). The desmoplakin component present in myocardial tissue has shown a similarly intense reaction and appeared in a similar position on two-dimensional gel electrophoresis. Fig. 2 also indicates that desmoplakins from different sources do not differ considerably in electrical charge from one tissue to another (compare, e.g., Fig. 2, a and b with data of reference 10). In such pH gradient gel electrophoreses, desmoplakin II

(DP1 and DP2, desmoplakins I and II). For the designation of polypeptide bands see reference 10. Lane b, Ponceau-S staining of protein after transfer to nitrocellulose paper. Lanes c-f, autoradiographs of parallel strips showing the reaction of antibodies DP1&2-2.15 (lane c), DP1-2.17 (lane d), antibody DP1-2.21 (lane e), antibody DP1&2-1.1 (lane f), using ¹²⁵I-labeled goat anti-mouse Ig to detect the binding of desmoplakin antibody.

TABLE I. Reactivity of Monoclonal Antibodies to Desmoplakins

A. Species specificities of antibodies to desmoplakins

Species	Antibodies*						
	DP1&2-2.15	DP1-2.17	DP1-2.6	DP1&2-2.2	DP1-2.20	DP1-2.21	DP1&2-1.1
Cow	+	+	+	+	+	+	+
Man	+	+	+	+	-	-	-
Rat	+	+	+	-	+	-	-
Chick	+	+	+	-	-	-	-

B. Desmoplakins identified in different bovine and human tissues by reaction with antibodies to desmoplakins†

Tissues‡	Desmoplakin I	Desmoplakin II
Stratified epithelia		
Epidermis		
Tongue		
Esophagus	+	+
Cornea		
Pseudostratified epithelium		
Trachea	+	+
Transitional epithelium		
Bladder	+	+
Heart		
Myocardium	+	-
Purkinje fibers		
Simple epithelia		
Liver		
Intestine	+(+)‡	-
Endometrium		
Cultured cell lines		
MDBK		
BMGE		
MCF-7	+	-
A-431		
TR-146		

* DP, desmoplakin; 1 and 2 refer to the polypeptide specificity; subsequent numbers give the epitope designation.

† In Results from experiments with antibodies DP1&2-2.15, DP1-2.17, and DP1-2.6, using immunoblot tests and immunofluorescence microscopy.

‡ Additional tissues examined include, for stratified epithelia, exocervix and human penile mucosa; for pseudostratified epithelia, human bronchial epithelium; for simple epithelia, human intestine, uterine endometrium and oviduct.

§ (+), antibody DP1-2.6 gives very weak staining on simple epithelia.

has frequently been found to migrate somewhat ahead of desmoplakin I. At present we cannot determine whether this reflects a slight difference in charge or is simply due to the lower molecular size. The fact, however, that both desmoplakins appear at nearly the same pH value on isoelectric

focusing (for examples see references 7 and 10) argues for the latter.

We have compared the desmoplakins from various stratified tissues with the corresponding proteins of cultured bovine cells by co-electrophoresing [³⁵S]methionine-labeled cytoskel-

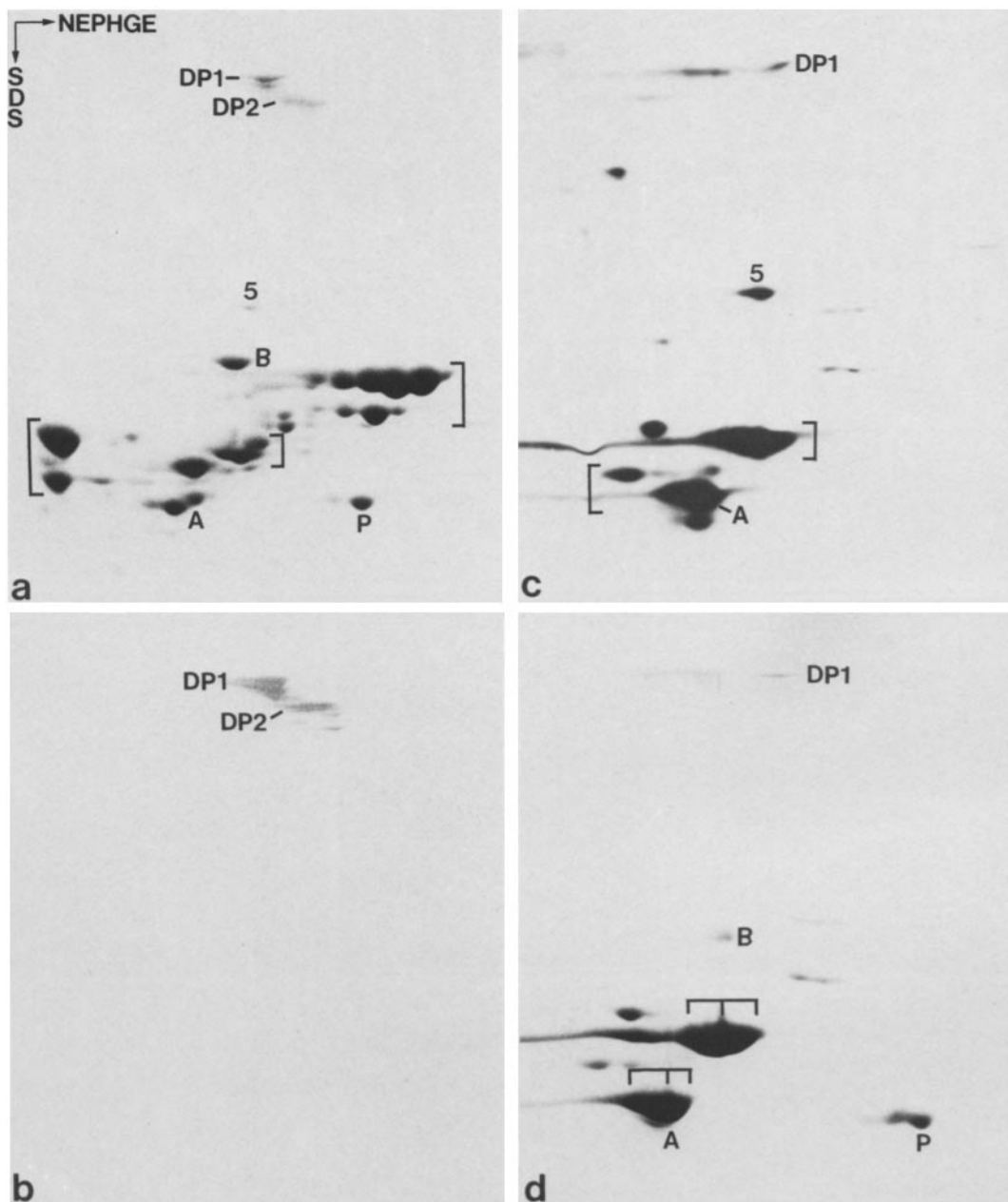


FIGURE 2 Identification of desmoplakin polypeptides by blotting of polypeptides separated by two-dimensional electrophoresis and a comparison, by co-electrophoresis, of desmosomal plaque proteins from stratified epithelia with those present in the cytoskeletal preparations from a cultured epithelial cell. Nonequilibrium pH gradient electrophoresis (NEPHGE) was used in the first dimension (basic polypeptides to the right) and electrophoresis in the presence of SDS in the second dimension. Markers used for co-electrophoreses are actin (A), bovine serum albumin (B), and phosphoglycerokinase (P). (a) Coomassie Blue staining of cytoskeletal proteins of bovine cornea. The positions of desmoplakins I and II and of band 5 are denoted by DP1, DP2, and 5, respectively. Brackets indicate the positions of the major cytokeratins (for details see reference 28). (b) Polypeptides of bovine cornea cytoskeleton separated on a gel parallel to that shown in a were transferred to nitrocellulose paper then reacted with antibody DP1&2-1.1 followed by staining with peroxidase-coupled rabbit anti-mouse Ig. Positive polypeptides show similar mobilities to those of desmoplakins I and II in a and also those of bovine snout epidermis (not shown; see reference 10). (c) Autoradiograph showing the co-electrophoresis of polypeptides of bovine cornea cytoskeleton (as in a) with those of a cytoskeletal preparation from [³⁵S]methionine-labeled BMGE-H cells. The positions of labeled polypeptides with mobilities similar to those of desmoplakin I and band 5 protein from cornea are indicated. Brackets denote the major cytokeratins of this cell line (for details see reference 33). (d) Coomassie Blue staining of a gel showing cytoskeletal proteins of BMGE-H cells.

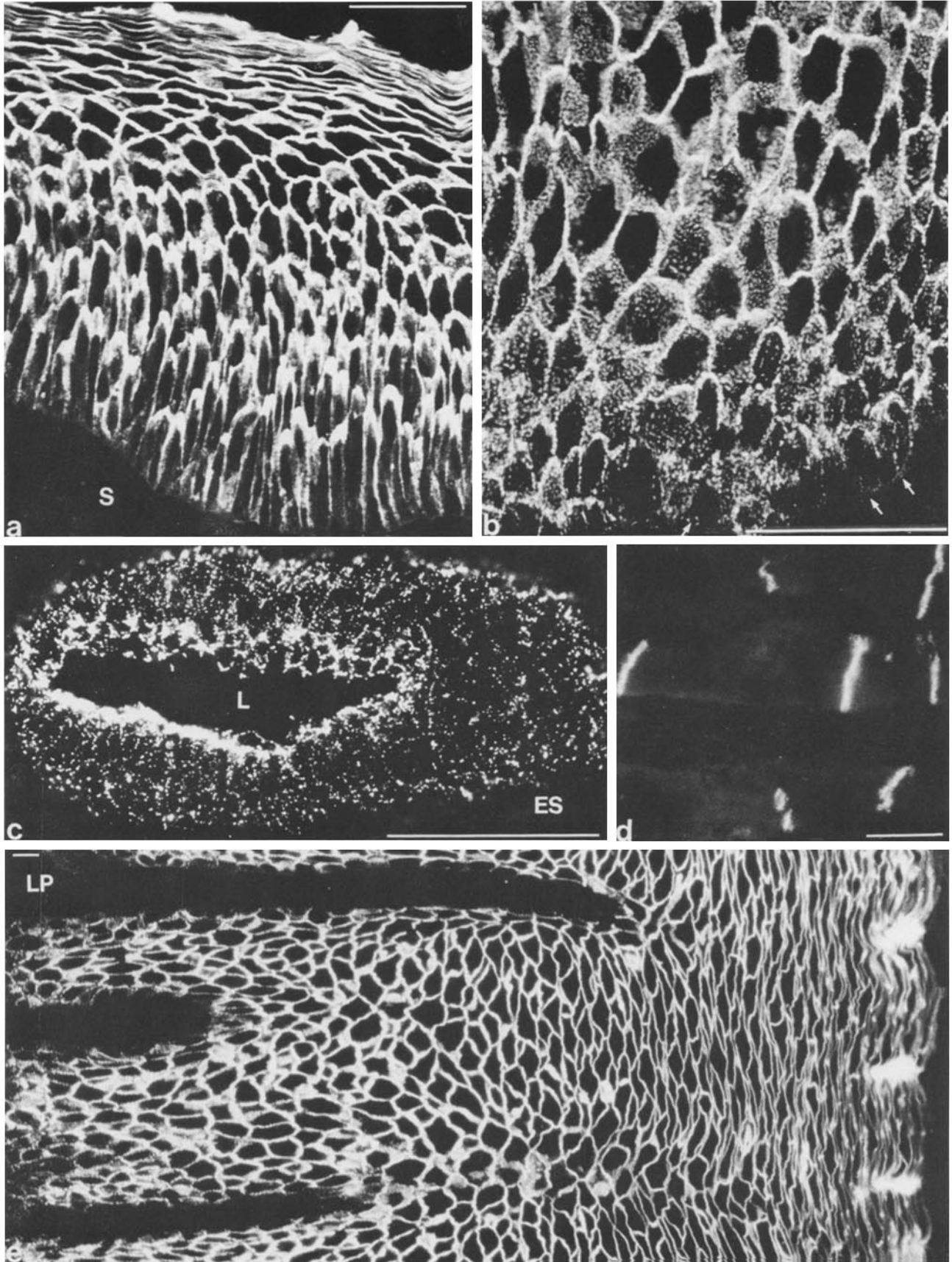


FIG. 3

etal proteins from cultured bovine mammary gland epithelial cells (BMGE – H and BMGE + HM) with desmosomal and/or total cytoskeletal proteins from bovine snout epidermis (not shown here; for data see reference 7) or cornea (Fig. 2, *a* and *c*). On such co-electrophoreses, desmoplakin I and band 5 protein from the cultured cells appear in similar positions as desmoplakin I and band 5 protein from the stratified tissues (Fig. 2*a*). Frequently, we have noticed that in the pH gradient, BMGE cell desmoplakin I migrates slightly faster than the protein from the stratified tissues. So far we cannot decide whether this reflects a true cell type-specific difference. Clearly the relative amount of desmoplakin is usually much lower in the cultured cells compared with the stratified tissues (Fig. 2*d*).

Positive staining of plasma membranes (Fig. 3*a*), which may be resolved at higher magnification into typical punctate patterns between epithelial cells and along their basal membranes (see arrows in Fig. 3*b*), has been found with each of the antibodies in all bovine tissues (Fig. 3, *a*, *b* and *e*; see Table I) known to contain true desmosomes, including intercalated discs of myocardium (Fig. 3*d*) and intercellular borders between Purkinje fiber cells (not shown). It is also noteworthy that antibodies specific for desmoplakin I stain all layers of stratified tissues (Fig. 3*e*). Positive reaction has also been obtained on human (Fig. 3*c*), rat, and chick tissues (Table I) with antibodies recognizing these species. All monoclonal antibodies gave the typical fluorescent dotted line appearance on cell borders of monolayer cultures of desmosome-containing epithelial cell lines. A typical example is shown in Fig. 4*a* on human MCF-7 cells with antibody DP1&2-2.15. In addition, some fluorescent dots can be seen in variable frequencies in intracytoplasmic positions (e.g., Fig. 4, *a* and *b*), representing either hemidesmosomes or desmosome-derived endocytotic vesicles (see references 12 and 40). Some cell lines such as BMGE – H, a bovine mammary gland-derived cell line, frequently show long continuous lines of staining along cell borders (Fig. 4*b*). Electron microscopy has confirmed the appearance of extremely long (giant) desmosomes in such cells (Fig. 4, *c* and *d*) that appear to be formed by the fusion of many normal-sized desmosomes, as a closer examination has revealed small regions of interjunctional cell membrane (see arrows in Fig. 4*c*) within such giant desmosomes.

Immunoelectron microscopy has shown these monoclonal antibodies to specifically react with the plaque region of desmosomes. Typical examples are shown on the very small desmosomes of the corneal epithelium (Fig. 4, *e*–*g*) and the relatively large desmosomes of bovine tongue mucosa (Fig. 4*h*) and confirm our previous peroxidase localization with conventional antisera to desmoplakins (10, 17, 18, 41).

Cell-Type Differences of Desmoplakin Composition

Cytoskeletal fractions of various tissues from bovine, human, and rat sources have been prepared and their proteins separated by SDS PAGE (Fig. 5). On immunoblotting, antibodies recognizing both desmoplakins I and II of cow snout desmosomes (e.g., DP1&2-2.15) react specifically, in all stratified tissues of the species examined, with polypeptides of mobilities identical to those of the snout polypeptides. These include bovine tongue, cornea, and esophagus as well as rat tongue, human epidermis, and lingual mucosa. Positive reaction is also seen with both polypeptides in the pseudostratified tissues of bovine (Fig. 5*a'*, lane 5') and rat trachea as well as transitional epithelium of bovine bladder (see Table I). In several cases the desmoplakins represent minor components, as judged from the Coomassie Blue-stained gels.

In contrast, heart tissue from all species shows only one positively reacting polypeptide that co-migrates with desmoplakin I of the stratified tissues (Fig. 5*a'*, lane 8', and Fig. 5*b'*, lane 3'). On co-electrophoresis, desmoplakin I of rat tongue and heart display the same mobility. Immunoblots of simple epithelia, i.e., bovine liver and rat intestine, also show reaction with a single polypeptide co-migrating with snout epidermal desmoplakin I (for an example see Fig. 5*a'*, lanes 9' and 10'). Furthermore, for all cell lines examined including examples of cells derived from both simple (BMGE – H and BMGE + HM; Fig. 5*a'*, lanes 6' and 7'; MCF-7, Fig. 5*c*, lane 5') and stratified (A-431 and TR-146; Fig. 5*c'*, lanes 3' and 4') tissues, all growing in monolayer, have presented only one positively reacting band that also co-migrates with bovine epidermal desmoplakin I. In all of these cases, appreciable reaction of a polypeptide of an electrophoretic mobility similar to that of desmoplakin II has not been detected with either monoclonal or conventional antibodies on heavily overloaded gels. The correspondence of the higher molecular weight polypeptide to desmoplakin I has been confirmed in all cases by blotting with antibodies specific for this polypeptide (e.g., DP1-2.17 and DP1-2.6). These antibodies have also revealed that bands which in some samples may appear in positions similar to that of desmoplakin II are in fact proteolytic products from desmoplakin I. To exclude the possibility that desmoplakin II might be lost as a result of proteolytic breakdown during the isolation procedure, BMGE – H and BMGE + HM cells have been boiled directly in SDS-containing sample buffer and their total proteins analyzed by SDS PAGE. Again only one band has been found to be positive upon immunoblotting of such material (an example is shown in Fig. 5*a*, lane 6'), indicating that the lack of desmoplakin II in blots of cytoskeletal fractions is genuine and does not

FIGURE 3 Typical examples of immunofluorescence microscopy are shown with monoclonal antibodies to desmoplakins (antibody DP1&2-2.15 and DP1-2.16) on 5- μ m frozen sections of various tissues. (*a*–*c*) Reaction with antibody DP1&2-2.15 recognizing both desmoplakins. (*a*) Bovine cornea, showing specific staining of cell boundaries of all layers. No staining is found in the underlying stroma (*S*). (*b*) Bovine tongue mucosa shown in grazing section, the punctate nature of the staining. Note also the staining of the hemidesmosomes located at the basal surface of the germinative layer, connecting them to the underlying basal lamina (arrows). (*c*) Endometrial epithelium of human uterus, showing dotted staining between the cells and intense staining in the subapical region toward the lumen (*L*), reflecting the higher concentration of desmosomes in the ring of maculae adherentes of this simple epithelium. Endometrial stroma (*ES*) is negative. (*d* and *e*) Reaction with antibody DP1-2.6 recognizing only desmoplakin I. (*d*) Positive reaction on the intercalated discs of bovine heart. (*e*) Positive reaction of the cellular boundaries in all layers of the stratified bovine tongue. Bars: (*a*–*c*) 50 μ m; (*d* and *e*) 20 μ m.

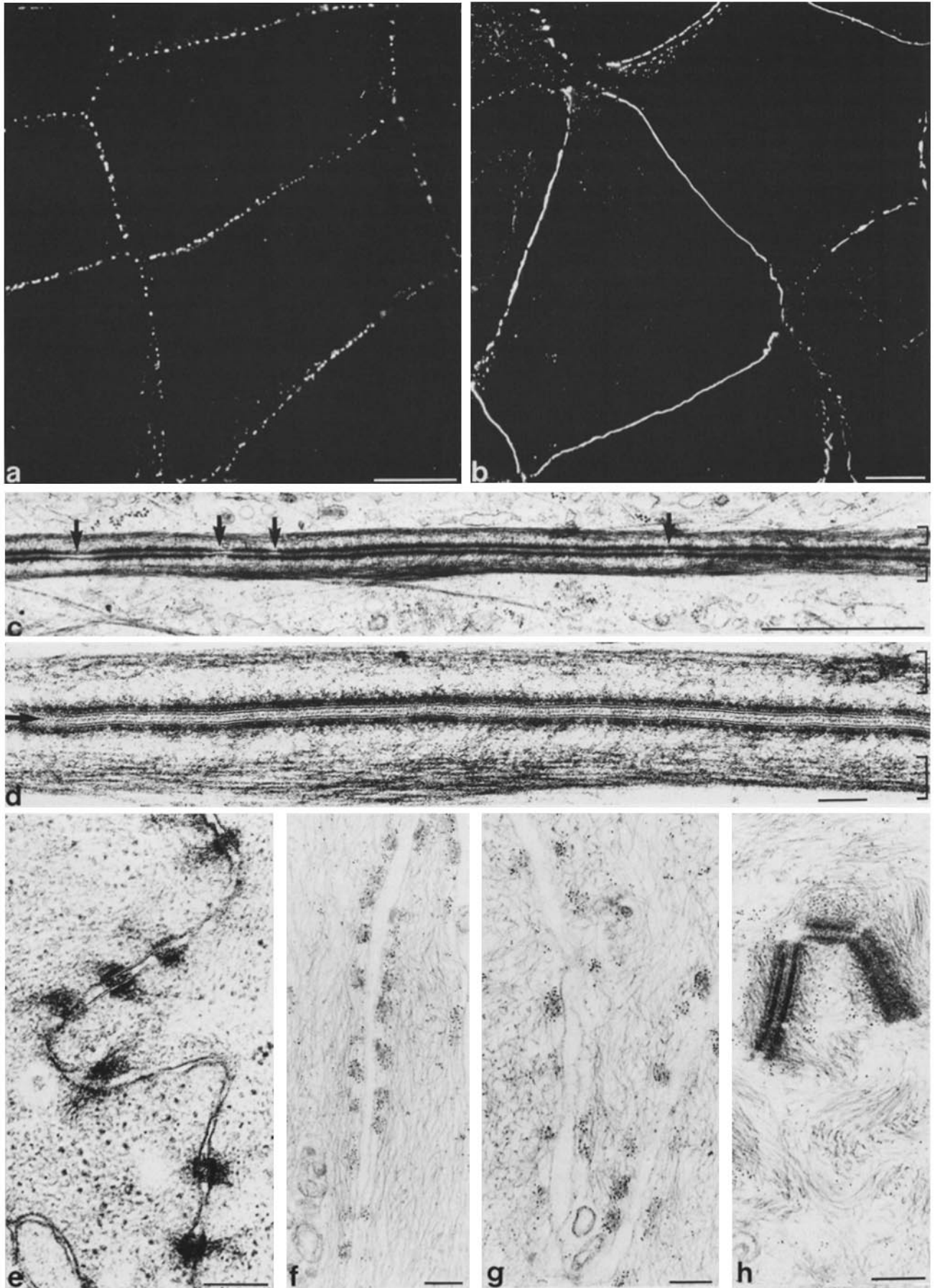


FIG. 4

result from the extraction procedure. Conversely, sections of snout epidermis boiled directly in SDS-containing sample buffer always show two desmoplakin bands, arguing against the possibility that desmoplakin II is produced as a proteolytic breakdown product during the isolation procedure (not shown; see references 10 and 42). With cultures of human keratinocytes, which readily stratify in culture, we have obtained inconsistent results. In some cultures, only desmoplakin I has been identified, whereas other cultures have presented a small amount of desmoplakin II in addition to I.

Identification of Band 5 Protein in Different Cell Types

Antibodies that react specifically with band 5 protein on purified preparations of cow snout desmosomes have been used to immunolocalize the cross-reacting component in plasma membranes and, specifically, the desmosomal plaques by fluorescence microscopy (Fig. 6) and immunoelectron microscopy (not shown). Typical desmosomal staining is seen in all epithelial cells, including examples of stratified and simple epithelia, intercalated discs of heart tissue, and cultured epithelial cells (Fig. 6, *a-e*).

On preparations of desmosome-tonofilament complexes prepared by the pH 9 method (5), we have noted that, in addition to the strongly reacting band 5 polypeptide of M_r 83,000, two minor bands of M_r 160,000 and \sim 300,000 react with these antibodies (Fig. 7*a'*, lane 2'); these probably result from the presence of cross-linked products (possibly dimers and tetramers). No reaction is seen on the cytokeratins which are present in very large amounts in these preparations (Fig. 7*a*, lane 2'). On all cell types examined, which include simple and stratified epithelia as well as myocardium of cow and rat, human tongue mucosa, and various cell culture lines of bovine and human origin, the antibodies react with a polypeptide co-migrating with snout band 5 protein (Fig. 7, *a-e*; Table I). The close similarity, if not identity, of the band 5 protein of stratified tissues to the antibody-binding polypeptide present in cell cultures derived from simple epithelia is further demonstrated by the co-electrophoresis of a polypeptide of identical charge and mobility on two-dimensional gel electrophoresis (cornea and BMGE - H cells are shown as typical examples in Fig. 2, *a*, *c* and *d*; for other stratified tissues see also reference 10).

DISCUSSION

Our results show that immunologically related polypeptides of similar molecular weights and electrical charges occur in

the desmosomal plaques of all the diverse cell types examined, which include examples of simple, stratified, pseudostratified, and transitional epithelia as well as representatives of nonepithelial cells, i.e., myocardial and Purkinje fiber cells of the heart, and various cultured cell lines. This indicates that the desmosomal plaque proteins of different cell types and species are biochemically very similar, if not identical, and are relatively stable throughout evolution. Our data further show that desmosomes that differ greatly in size (e.g., cornea and tongue in Fig. 4, *e-h*), substructural appearance (2, 22, 34, 43, 44), and in the specific type of intermediate filament attached to the plaques (e.g., desmin in myocardium, cytokeratins in epithelia) all contain common plaque proteins, with the exception of desmoplakin II which will be discussed below. Therefore, we conclude that the desmosomal plaque is formed, in these diverse cell types, by a distinct set of proteins that defines a certain kind of cell, the desmosome-forming cells, a category of cell differentiation not congruent with other known programs of expression.

The identification of the plaque proteins which, in many cases, represent minor cell components in the different cell types, has been made possible by the availability of a panel of highly specific monoclonal antibodies to desmosomal plaque proteins some of which recognize epitopes common to the diverse species examined. Therefore, these antibodies should also provide excellent probes to study desmosome formation in embryogenesis and also to characterize certain groups of tumors (see reference 41).

We have identified desmoplakin I to be a large polypeptide (M_r 250,000) localized exclusively to the desmosomal plaques in all cell types containing true desmosomes. Both its apparent molecular weight and isoelectric charge, which is near neutrality, are very similar in the different cell types, including cultured epithelial cells and myocardial tissue. These results extend our previous observations (10, 17) and those of Steinberg's laboratory (24) on some stratified epithelia and myocardium. We now conclude that desmoplakin I is an obligatory constituent of the desmosomal plaque and that antibodies to it can serve as a general probe for the identification of desmosomes, including desmosome-derived intracytoplasmic vesicles (2, 12, 40), and to distinguish them from other plaque-bearing membrane domains (see reference 45). Our data also demonstrate that desmoplakin I contains portions conserved during evolution as well as other epitopes that have diverged among the various species examined.

However, this is not the case for desmoplakin II, a polypeptide of M_r 215,000, which is very similar in isoelectric pH value and tryptic peptide map to desmoplakin I and also

FIGURE 4 Immunofluorescent staining of monolayer cultures of human MCF-7 cells (*a*) and bovine BMGE - H cells (*b*), showing the typical punctate staining at the cell borders and occasional spotted staining within the cell which represent either hemidesmosomes or intracellularly accumulated desmoplakin-associated vesicles (17, 42). In addition long stretches of uninterrupted staining are often seen which correspond to the electron microscopic appearance of giant desmosomes often found in the BMGE - H cells (*c* and *d*). These appear to result from the fusion of many smaller desmosomes. Membrane and midline structures are resolved at higher magnification (*d*); horizontal arrow denotes the midline plate. Small regions of interdesmosomal membrane within these fused desmosomes are marked by small arrows (*c*). Brackets indicate the bundles of cytokeratin filaments that mostly run parallel to the desmosomal plaques. (*e*) Electron micrographs of bovine cornea, showing the presence of numerous very small desmosomes located along the convoluted plasma membranes of adjacent cells. Immunoelectron microscopic location of staining with antibodies DP1&2-2.15 (*f*) and DP1&2-1.1 (*g*), respectively, by 5-nm gold-coupled goat anti-mouse IgG. The gold particles are concentrated on the desmosomal plaques. (*h*) Immunogold localization on plaques of the larger desmosomes of bovine tongue mucosa. Note the restriction of label to the periphery of the plaque which reflects difficulties of the penetration of gold-Ig complexes to the deeper regions of the plaque; for plaque staining by the immunoperoxidase technique see references 10, 17, and 18. Bars: (*a* and *b*) 10 μ m; (*c*) 1.0 μ m; (*d*) 0.1 μ m; (*e-h*) 0.2 μ m.

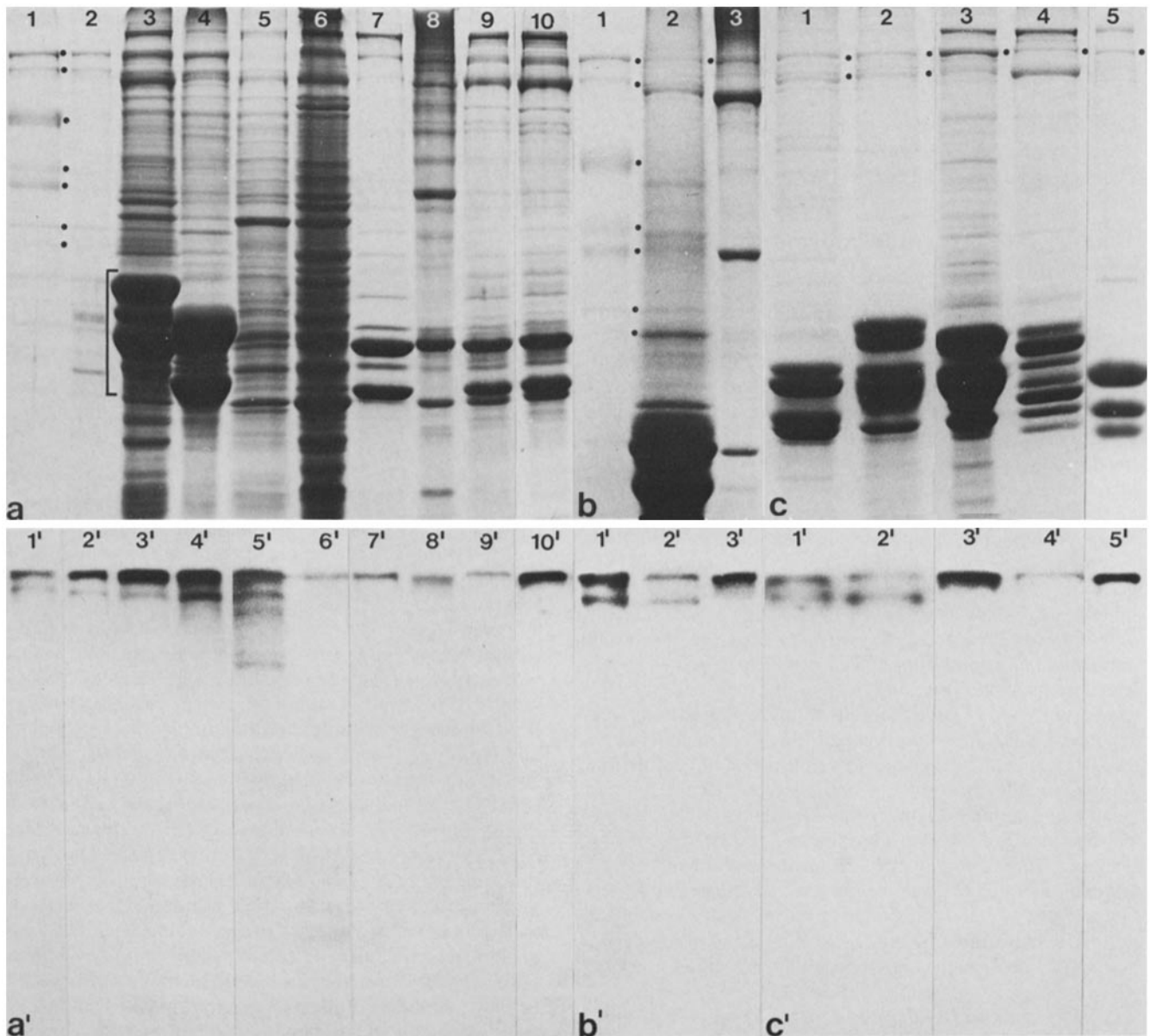


FIGURE 5 Detection of desmoplakins in various bovine cells by immunoblotting. (a) Coomassie Blue staining of proteins after SDS PAGE. Lane 1, purified snout desmosomes; dots mark from top to bottom the positions of desmoplakins I and II, band 3, bands 4a and 4b, band 5, and band 6. Lane 2, desmosomal fraction of tongue. Cytoskeletal fractions of: cornea (lane 3; brackets indicate the large amounts of cytokeratins in such preparations; esophagus (lane 4); trachea (lane 5), BMGE – H cells (lane 7); heart (lane 8); liver (lanes 9 and 10); total cellular proteins of intact BMGE – HM cells (lane 6). (a') Autoradiograph of the corresponding immunoblot reaction with antibody DP1&2-2.15. Note the presence of two desmoplakin bands in the stratified (lanes 1'–4') and the pseudostratified (lane 5') tissues. Only one desmoplakin band is detected within the cultured BMGE – HM (lane 6') and BMGE – H (lane 7') cells and heart tissue (lane 8') as well as the representative of simple epithelium, i.e., liver (lane 9'). Even after extensive loading of the liver sample, only one band can be detected by the antibody (lane 10'). The slightly higher mobility of the band in heart (lane 8') is probably due to the large amounts of protein in this region of the gel. In parallel analyses the upper bands have also been found positive with DP1-2.17 and DP1-2.6, identifying them as desmoplakin I. (b) Identification of desmoplakins in rat tissues. Coomassie Blue-stained gel showing proteins of bovine snout desmosome-enriched fractions (citric acid method) analyzed by SDS PAGE (lane 1; dots as previous figures), a cytoskeletal fraction of rat tongue mucosa (lane 2), and a cytoskeletal fraction of rat heart (lane 3). (b') Autoradiograph of the corresponding immunoblot reaction with monoclonal antibody DP1&2-2.15. The reaction was visualized by ^{125}I -labeled goat anti-mouse Ig. Two bands were found in the bovine snout epidermis (lane 1') and rat tongue mucosa (lane 2'). However, the rat heart shows only desmoplakin I (lane 3'), identified as such by DP1-2.6 (not shown). (c) Coomassie Blue-stained gel showing proteins of several human tissues: Cytoskeletal fractions of human tongue mucosa (lane 1) and human epidermis (lane 2) are compared with cytoskeletal fractions of cultured A-431 (lane 3), TR-146 (lane 4), and MCF-7 (lane 5). (c') Corresponding autoradiograph showing immunoblot reaction with monoclonal antibody DP1&2-2.15 visualized with radioactively labeled goat anti-mouse Ig as secondary antibody. Again, two bands are detected in the stratified (lanes 1' and 2') but only one in each of the cultured cell lines that are derived from stratified (lanes 3' and 4') and simple tissues (lane 5').

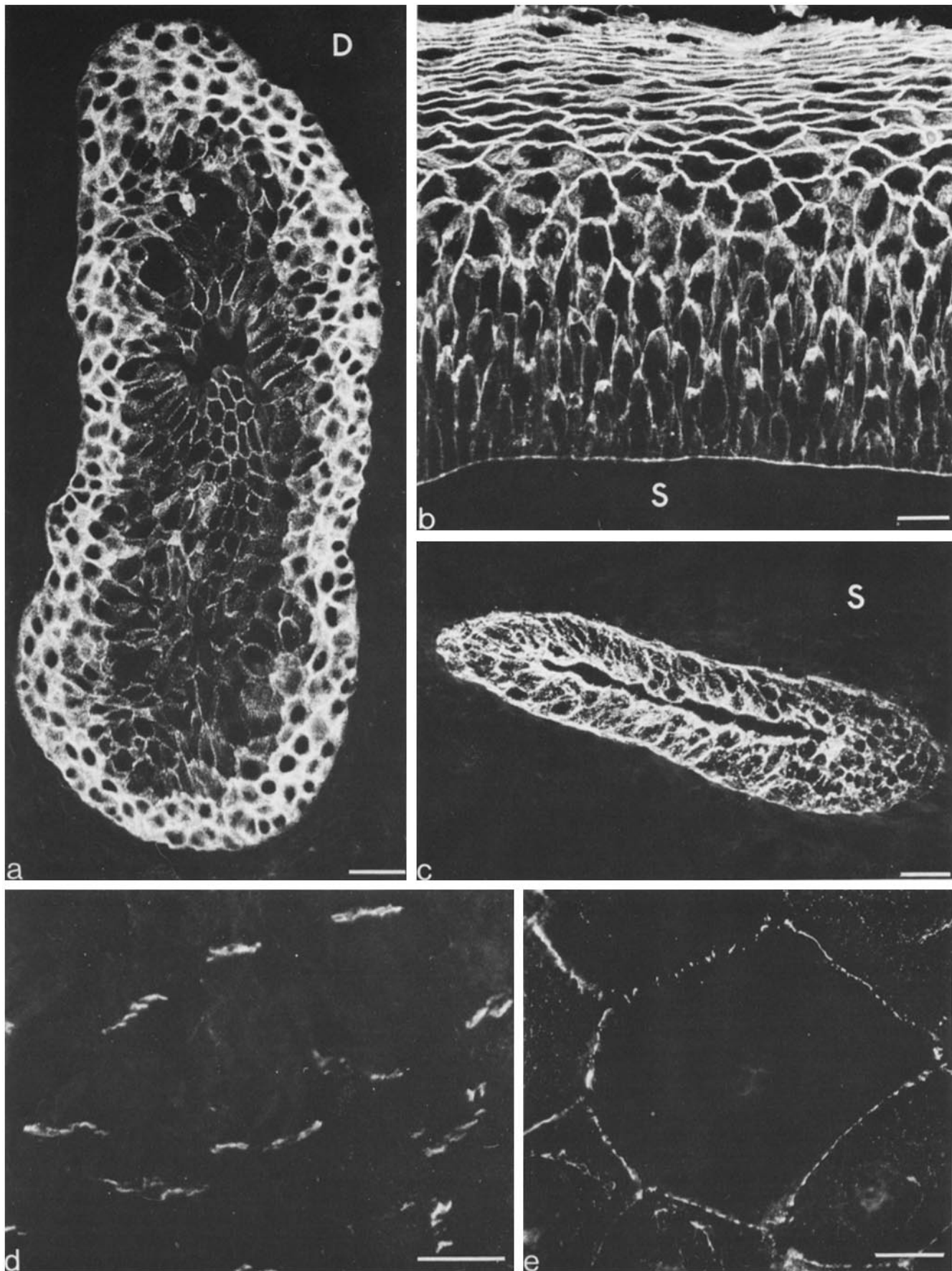


FIGURE 6 Immunofluorescence microscopy showing the desmosomal location of antibodies to band 5 protein on frozen sections of the glandular ducts of the bovine snout (a); note punctate staining in some regions. (b) Bovine cornea; note the staining of cell boundaries of all layers including the basal surface of the germinative cells. (c) Human endometrial ducts. (d) Bovine heart; note specific staining of intercalated discs, cultured BMGE – H cells (e). Cells of stroma (S) and dermis (D) are negative in all cases. Bars, 50 μ m.

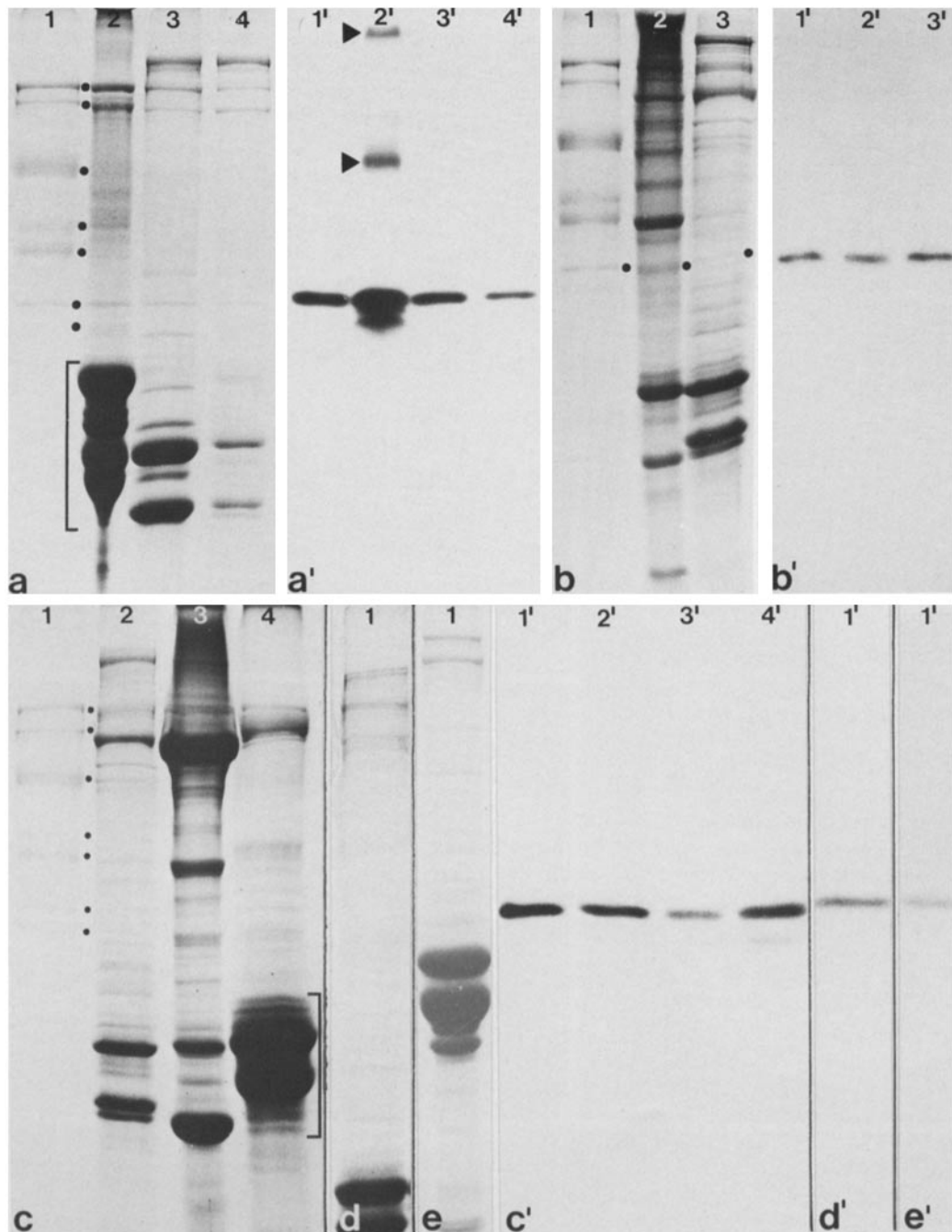


FIGURE 7 Immunological identification of desmosomal band 5 protein in diverse cell types. (a) Coomassie Blue-stained gel showing SDS PAGE-separated proteins of bovine snout desmosome fractions prepared according to the citric acid method (lane 1; dots denote the major desmosomal polypeptides as in previous figures), bovine snout desmosome-tonofilaments fraction prepared according to the pH 9 method (lane 2; brackets denote cytokeratins which are abundant in such preparations), cytoskeletal fractions of: BMGE - H cells (lane 3), and BMGE + HM cells (lane 4). (a') Autoradiograph of the corresponding immunoblot reaction with guinea pig antiserum to band 5 protein visualized by incubation with ^{125}I -protein A. A single band corresponding to M_r 83,000 is evident in the citric acid desmosome preparation and in the cytoskeletal extracts; however, in pH 9 fractions of snout desmosomes two additional minor bands of higher molecular weights that probably represent dimers and tetramers of band 5 protein also react with the antibodies (arrowheads in lane 2'). Minor positive components of slightly higher mobility than band 5 protein are also detectable in lane 2' probably are the result of proteolytic breakdown. No reaction is found on the cytokeratins which are greatly abundant in such preparations (compare a, lane 2, brackets). (b) Coomassie Blue staining of polypeptides separated by SDS PAGE showing components of bovine snout desmosomes (lane 1) in comparison with cytoskeletal material of bovine heart (lane 2) and bovine liver (lane 3). Spots in all lanes mark the position of band 5 protein. (b') Autoradiograph of a corresponding immunoblot of a parallel gel showing the reaction with antibodies to band 5 protein visualized by binding of ^{125}I -protein A. Note that only one band co-migrating with the epidermal band 5 polypeptide reacts in all samples. (c) Identification of desmosomal band 5 protein in diverse bovine, rat, and human tissues by immunoblotting of proteins separated by SDS PAGE with specific guinea pig antibodies. (a) Coomassie Blue-stained gel of bovine snout desmosome fraction (lane 1; dots designate polypeptides as in previous figures), bovine liver cytoskeletal fraction (lane 2), cytoskeletal fractions of rat heart (lane 3), rat tongue (lane 4; brackets denote cytokeratins). (d and e) Coomassie Blue-stained gel of rat liver (d) and A-431 (e) cytoskeletal fractions. (c'-e') Corresponding autoradiograph of immunoblot, showing exclusively reaction of a single polypeptide of M_r 83,000 in all tissues and cell cultures (visualization of the immunoreaction by binding of ^{125}I -protein A).

shares certain immunological epitopes with it (this study and references 8 and 10). Clearly, desmoplakin II differs from desmoplakin I in several epitopes as we have found a number of different monoclonal antibodies that are unique to desmoplakin I. Whether this reflects true sequence heterogeneity or simply the absence of a sizeable portion in a proteolytic product from desmoplakin I cannot be decided at present (see also reference 8).

We have found the quantity of desmoplakin II in relation to desmoplakin I to vary considerably between the different cell types (Fig. 5). Furthermore, desmosomes of the myocardial intercalated discs, a nonepithelial tissue, and those of simple epithelia and various cultured cells derived from either simple or stratified epithelia, appear to lack desmoplakin II. No trace could be found either on Coomassie Blue-stained, heavily loaded gels or by immunoblotting. Although we cannot exclude the possibility that desmoplakin II is lost during the preparation, we regard this as rather unlikely because we have also not detected desmoplakin II after boiling of intact cells directly in SDS-containing sample buffer, which minimizes proteolysis by immediate denaturation of the proteins. Therefore, we conclude that desmoplakin II is not expressed in appreciable quantities in simple epithelia, in myocardial cells, and in several cultured cell lines growing in monolayer.

In contrast, we have found considerable amounts of a protein corresponding to bovine epidermal desmoplakin II in all stratified epithelia, in the pseudostratified epithelia of the respiratory tract, and in the transitional epithelium of the bladder. This is contrary to a recent report of Giudice et al. (24) who could not detect desmoplakin II in any tissue with the exception of epidermis. This, however, could be due to their isolation methods which may tend to lose selectively plaque proteins (10, 18). Therefore, our results indicate that desmoplakin II is a protein produced under some form of cell type-specific regulation related to the stratification process. In this context it is of note that several cell lines of stratified epithelial origin such as A-431 and TR-146, which normally do not stratify in culture, also lack desmoplakin II. Whether the low level of desmoplakin II band which we have observed in some cultures of human keratinocytes, but not in all, is related to the specific degree of stratification in the culture remains to be examined.

While our immunocytochemical observations have clearly shown that desmoplakin I is present in all layers of stratified epithelia, we cannot exclude the possibility that desmoplakin II is heterogeneously distributed across such epithelia, a possibility that escapes detection when pooled tissue material is used (for discussion see also reference 42). Clearly, antibodies specific to desmoplakin II would be of great value in deciding this question.

The polypeptide band 5 of bovine epidermal desmosomes, which has an M_r value of 83,000 and, on two-dimensional gel electrophoresis, is almost isoelectric to serum albumin, is chemically and immunologically different from the desmoplakins. It is encoded by a specific mRNA and, like the desmoplakins, is not significantly glycosylated (11). Mueller and Franke (10) have described a polypeptide of similar size and isoelectric pH in tongue mucosa, and Steinberg and colleagues (8, 24) have reported an immunologically cross-reacting polypeptide of this size in two other stratified epithelia, i.e., cornea and esophagus. Our present results confirm the existence of this protein in stratified epithelia and further show that it occurs generally wherever desmosomes occur,

i.e., in transitional, pseudostratified, and simple epithelia, various kinds of cultured epithelial cells, and in the intercalated discs of myocardial tissue. This is in agreement with previous immunofluorescence microscopy of Cowin et al. (19, 20, 22). Thus, we conclude that band 5 protein is a general desmosomal plaque constituent which in all cells examined so far is co-expressed with desmoplakin I.

From our study a remarkably uniform picture of the composition of the desmosomal plaque emerges. Desmosomes of all cell types examined so far have two major proteins in common: desmoplakin I and band 5 protein. Both are non-glycosylated proteins contributing to the formation of an insoluble protein polymer layer associated with the inner, i.e., cytoplasmic aspect of the desmosomal membrane domain. Whether both proteins are independently in contact with the membrane on the one hand and the specific kind of intermediate filaments on the other is not clear at the moment. Clearly, at least some desmoplakin I is freely accessible on the surface of the desmosomal plaque as shown by our finding that desmoplakin antibodies microinjected into cultured Madin-Darby bovine kidney cells specifically bind to the plaque (2). Future experiments will have to show the specific roles of desmoplakin I and band 5 protein in the mediation of membrane-intermediate filament interaction.

While the expression of desmoplakin I and band 5 protein is apparently correlated with desmosome formation, it is not necessarily coordinated with the expression of other cytoskeletal proteins. In fact, it is clear that expression of these two desmosomal proteins can be independent from the expression of cytokeratins and other epithelial proteins as well as from the expression program characteristic of myocardial cells. Thus the control of expression of these desmosomal plaque proteins does not seem to be linked to the synthesis of a specific type of the intermediate filament proteins. This independence of expression is also suggested by the absence of desmosomal plaques and desmosomal proteins in certain cultured epithelial cells such as those of the PtK₂ and various rat hepatoma lines (10, 22, 46). Work using cDNA cloning of desmosomal proteins is under way in our laboratory to provide adequate probes to study the regulation of expression of the various desmosomal plaque proteins.

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