A Protein Antigenically Related to Nuclear Lamin B Mediates the Association of Intermediate Filaments with Desmosomes

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Abstract. Desmosomes are specialized domains of epithelial cell plasma membranes engaged in the anchoring of intermediate filaments (IF). So far, the desmosomal component(s) responsible for this binding has not been unambiguously identified. In the present work, we have examined bovine muzzle epidermis desmosomes for the presence of protein(s) structurally and functionally related to lamin B, the major receptor for IF in the nuclear envelope (Georgatos, S. D., and G. Blobel. 1987. *J. Cell Biol.* 105:105–115). By using polyclonal antibodies to lamin B in immunoblotting experiments, we find that a desmosomal protein of 140-kD shares epitope(s) with lamin B. Immunoelectron microscopic and urea extraction experiments show that this protein is a peripheral protein localized at the cytoplasmic side of the desmosomes (desmosomal plaques). Furthermore, this protein binds vimentin in an in vitro assay. Since this binding is inhibited by lamin B antibodies, the epitopes common to the 140-kD protein and to lamin B may be responsible for anchoring of intermediate filaments to desmosomes. These data suggest that lamin B–related proteins (see also Cartaud, A., J. C. Courvalin, M. A. Ludosky, and J. Cartaud. 1989. *J. Cell Biol.* 109:1745–1752) together with lamin B, provide cells with several nucleation sites, which can account for the multiplicity of IF organization in tissues.

ASSOCIATION of intermediate filaments (IF)[1] with cellular membranes is a prominent feature of all eukaryotic cells (reviewed in Lazarides, 1980; Geiger, 1987; Steinert and Roop, 1988; Bershadsky and Vasiliev, 1988; Robson, 1989). However, progress in the molecular characterization of such interactions has been achieved only recently. In vitro recombination experiments of type III IF proteins (vimentin or desmin) with erythrocyte plasma membranes and nuclear envelopes have emphasized the role of two proteins. Ankyrin, a major constituent of the cortical skeleton, represents the capping site for the NH_2-terminal part of IF at the plasma membrane (Georgatos and Marchesi, 1985; Georgatos et al., 1985, 1987; Georgatos and Blobel, 1987a). A functionally distinct set of interactions takes place at the nuclear envelope where lamin B is the main receptor for the carboxy termini of IF proteins. Since this latter interaction is cooperative and non-saturable, lamin B likely represents a nucleation site for IF (Georgatos and Blobel, 1987a,b; Georgatos et al., 1987). Thus the structurally apolar IF have been proposed to acquire polarity via their nucleation on lamin B and capping on ankyrin.

Although not verified in vivo, this model is consistent with the observation that in mesenchymal, glial, and neuronal cells, the IF meshwork radiates from the nuclear periphery towards the cell surface. However, it may not be relevant to other cells where the IF network is organized differently. For example, in the electrocyte of *Torpedo* electric tissue, a flat syncytium that receives a dense cholinergic innervation on one of its surfaces, the desmin meshwork is anchored to the two opposite plasma membranes and is not organized around nuclei (Kordeli et al., 1986, 1987). Biochemical and immunocytochemical investigations have demonstrated that the plasma membranes of the innervated and the noninnervated sides of the cell, as well as the cortical skeleton associated with them, have a different composition. We have recently shown that desmin filaments are inserted to the innervated membrane via a lamin B–related protein of M_r ~54 kD, and to the noninnervated membrane via ankyrin (Kordeli et al., 1986; Cartaud et al., 1989). The postsynaptic membrane in rat muscle is also labeled with anti–lamin B antibodies (Cartaud et al., 1989). These observations suggest that the model of nucleation of IF based on nuclear lamin B may be generalized to other cell compartments. Accordingly, proteins structurally related to lamin B present in specialized domains of the plasma membrane may represent alternative or additional nucleation sites for the control of IF meshwork formation.

Another example of a peculiar IF organization is found in epithelial cells where extensive bundles of cytokeratin filaments contact the cell surface at specialized domains of the

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1. *Abbreviations used in this paper:* CASC, citric acid-sodium citrate, pH 2.6; IF, intermediate filaments.

plasma membrane called desmosomes (Farquhar and Palade, 1963). In rat mammary epithelial cells, the desmosomes also represent the principal organizing centers (i.e., nucleation centers) for cytokeratins (Bologna et al., 1986). These observations suggest that the desmosomes possess some unique IF binding and/or nucleation properties as compared with the nondesmosomal plasma membrane.

In this work, we have examined desmosomes for the presence of proteins structurally and functionally related to nuclear lamin B.

Materials and Methods

Anti-Lamin Antibodies

Human auto antibodies directed against lamin B (serum F) from a patient with systemic lupus erythematosus have been previously characterized (Guilly et al., 1987). They recognize lamin B in several species from mammals (Guilly et al., 1987) to fish (Cartaud et al., 1989; Cartaud, A., J. C. Courvalin, B. Jasmin, and J. Cartaud. 1989. *J. Cell Biol.* 109[4, Pt. 2]:133a[Abstr.]) and amphibian (Krohne et al., 1987). Another polyclonal antibody raised in rabbit against lamin B purified from *Torpedo* nuclear envelopes by SDS-PAGE was used.

Auto antibody to lamins A and C has been previously described (serum LS1) (McKeon et al., 1983).

Inhibition Experiments

Inhibition experiments were performed either by absorption of serum F (1:100) with lamin B purified on 10% SDS-PAGE from rat liver nuclear envelopes after partial renaturation (the 68-kD band excised from the gel was incubated at least 18 h in PBS containing 1% BSA and then homogenized) or with rat liver pore complex/lamina fractions (see below). In this latter experiment, we have verified that only the reactivity with epitopes corresponding to lamin B were removed from the serum (Guilly et al., 1987; Cartaud et al., 1989; Cartaud, A., J. C. Courvalin, B. Jasmin, and J. Cartaud. 1989. *J. Cell Biol.* 109[4,Pt. 2]:133a[Abstr.]). Both protocols resulted in total extinction of the signal either in immunoblotting of purified desmosomal fractions or in immunofluorescence experiments on muzzle epithelium.

Preparation of Pore Complex/Lamina Fractions

Pore complex/lamina fractions from rat liver were prepared according to Gerace et al. (1984) with slight modifications (Guilly et al., 1987). Briefly, minced rat livers were homogeneized in 2.2 M sucrose in 25 mM KCl, 5 mM MgCl₂, 50 mM Tris-HCl, pH 7.5 containing 1 mM PMSF, 10 mM N-ethyl-maleimide with a Chauveau homogeneizer (10 strokes). Nuclei were collected by sedimentation through a 30% sucrose cushion in the same buffer. These were then digested with DNase I (20 μg/ml) and RNase A (20 μg/ml) for 1 h at 4°C. A purified nuclear pore complex lamina fraction was obtained by further extraction of intrinsic proteins from nuclear envelopes with 0.3 M NaCl and 2% Triton X-100.

Preparation of Desmosomes

Desmosomes were isolated from bovine muzzle epidermis by a modification (Gorbsky and Steinberg, 1981) of the method of Skerrow and Matoltsy (1974). Briefly, 5 g of sliced bovine muzzle epidermis from freshly slaughtered animals were homogenized (Polytron Instruments type 20; Kinematica GmBH, Luzern, Switzerland) in 125 ml of CASC buffer (0.1 M citric acid-sodium citrate, pH 2.6), containing 0.05% NP-40 nonionic detergent, protease inhibitors leupeptin (5 μg/ml) pepstatin A (5 μg/ml) (Sigma Chemical Co., St. Louis, MO) 1 mM PMSF, and 1 mM DTT and then stirred for 3 h at 4°C. The homogenate was passed through a 50-μm nylon mesh and the filtrate was then centrifuged at 13,000 g for 20 min. The pellet was resuspended in CASC buffer containing 0.01% NP-40 and protease inhibitors, sonicated for 10 times at 15 s (Branson Sonic Power Co., Danbury, CT), and then centrifuged again at 13,000 g for 20 min. The desmosome-rich upper layer of the pellet was resuspended in CASC buffer and recentrifuged three times. The final pellet containing purified desmosomes was

resuspended in CASC buffer containing protease inhibitors and stored at −70°C until used.

Urea Extraction

Dissection of desmosomes into cytoplasmic protein and membrane glycoprotein domains was achieved according to Skerrow et al. (1987). Desmosomes stored in CASC buffer (see above) were diluted in 20 vol of distilled water containing protease inhibitors and centrifuged at 20,000 g for 20 min. Desmosomes (2.5 mg protein) were extracted for 30 min at 20°C with 5 ml of extraction buffer (20 mM Tris-HCl, pH 7.5, 25 mM β-mercaptoethanol, 1 mM PMSF, and 9 M urea). Control sample was extracted in the same condition without urea. Samples were centrifuged in polyallomer tubes at 200,000 g for 2 h at 20°C in a SW 50.1 rotor (Beckman Instruments, Inc., Palo Alto, CA). Supernatants were dialyzed against several changes of distilled water containing PMSF and lyophilized. Urea residues and extracts were subsequently dissolved in the same volume of SDS sample buffer (0.5 ml) to allow the relative comparison of the content of the proteins in each sample.

SDS-PAGE and Immunoblotting

Desmosomal components and urea extracts were separated on one-dimensional 8% SDS-PAGE according to Laemmli (1970) using a slab cell (Miniprotean II; Bio-Rad Laboratories, Richmond, CA) operating at 200 V for 45 min.

Two-dimensional gel electrophoresis was performed according to O'Farrell (1975) with some modifications (Mueller and Franke, 1983) because of the low solubility of desmosomal components in the first dimension (isofocussing) medium. Desmosomal proteins were solubilized by boiling for 5 min in the sample buffer of Laemmli (1970), then precipitated in cold acetone, dried, and finally treated with the urea-containing lysis buffer. Isoelectrofocussing gels were made in 2% ampholines 3,5-10, 2% ampholines 5-7, and 2% ampholines 7-9 for 4 h at 400 V and then 30 min at 800 V. Second dimension was carried out in 8% polyacrylamide gels containing 0.15% SDS.

Desmosomal proteins separated by one- or two-dimensional SDS-PAGE were transferred onto nitrocellulose paper (Towbin et al., 1979) and stained with Ponceau red. Immunoblots were revealed with alkaline phosphatase-conjugated goat anti-human or -rabbit immunoglobulins (1:5,000; Promega Biotec, Madison, WI) and the color developed with the 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium reaction.

Vimentin Binding Assay

Direct binding of purified bovine lens vimentin (Bloemendal et al., 1985) was achieved on 8% SDS-PAGE separated proteins transferred on nitrocellulose sheets (BA85 0.45 μm; Schleicher & Schull, Inc., Keene, NH) according to the "Western blotting" technique (Towbin et al., 1979). In all experiments, vimentin was preferred to cytokeratins for its relative solubility in aqueous solutions and for its high affinity for lamin B (Georgatos et al., 1987). Renaturation of proteins and blocking were achieved by a 48-h incubation of nitrocellulose strips at 20°C in PBS containing 5% dry skim milk. The strips were then left overnight in 50 mM Tris-HCl pH 7.4, 150 mM KCl, 1 mM EGTA, 1% dry milk, containing vimentin (∼1 μM) at 4°C. After washing (6 × 10 min), 1% dry milk, 0.2% Tween 20 in PBS, the strips were incubated in PBS containing mouse monoclonal anti-vimentin antibody (1:50, Boehringer Mannheim Biochemie, Mannheim, FRG) for 1 h at 20°C. After washing (six times for 10 min in PBS, 1% dry milk and 0.2% Tween 20), the strips were incubated with alkaline phosphatase conjugated goat anti-mouse IgG (1:2,000, Promega Biotec) for 30 min at 20°C. Finally, the strips were rinsed and the color reaction developed with 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium reaction. Control experiments were performed (a) without vimentin and (b) by substituting vimentin with unrelated cytoskeletal proteins such as α and β tubulins (∼1 μM) in the binding assay. Inhibition of vimentin binding was achieved by incubation of the strips with rabbit anti-lamin B antibody (1:100) before incubation with purified vimentin.

Immunofluorescence Microscopy

Cryostat sections (4-5 μm) of bovine muzzle epithelium were obtained according to Kordeli et al. (1986). Serum F (1:100–1:200 dilution) was used as first antibody and revealed with rhodamine-conjugated goat anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Stained
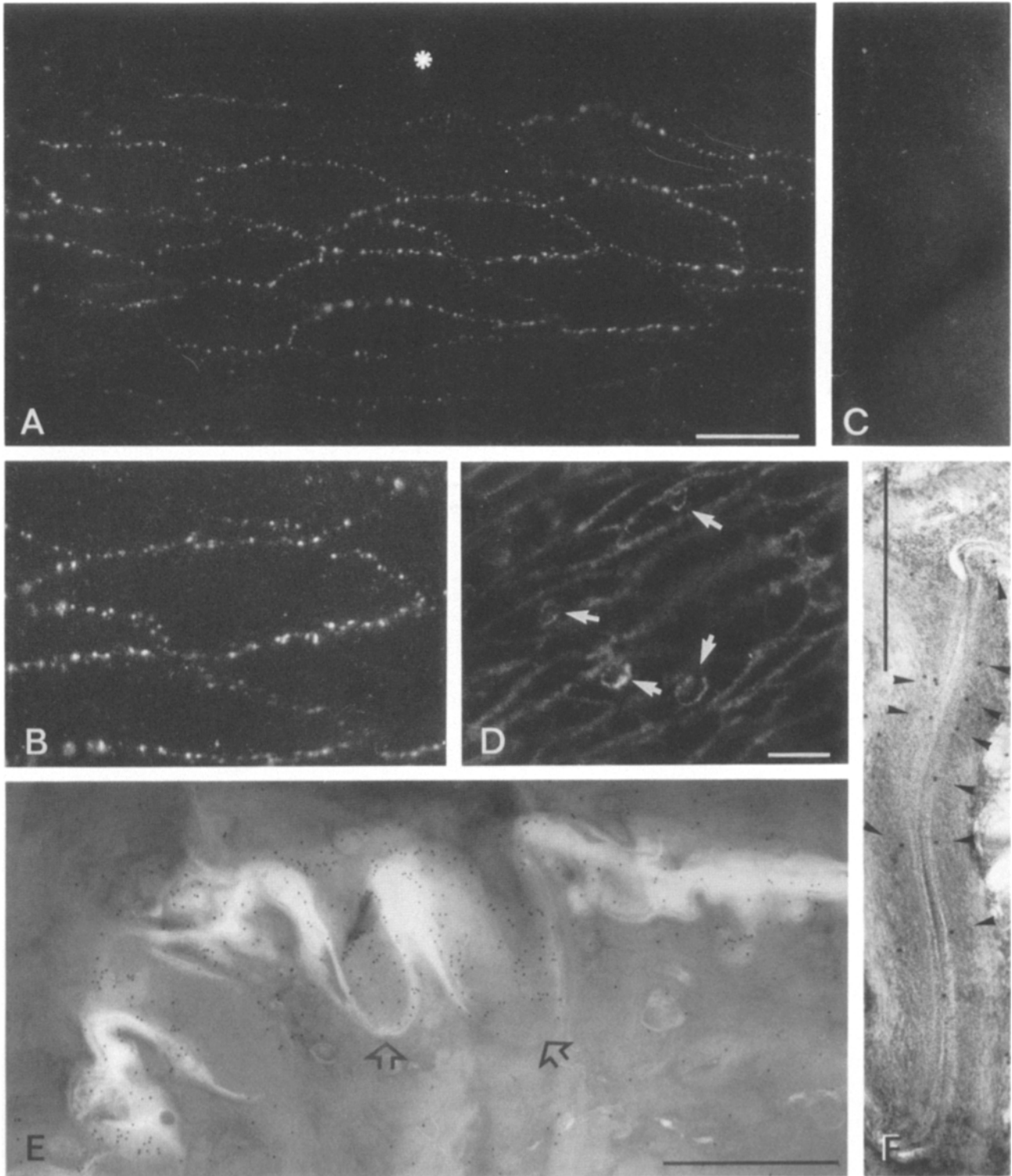
Figure 1. Immunocytochemical labeling of bovine muzzle epithelium with anti–lamin B antibodies (serum F). (*A–C*) Indirect immunofluorescence of a sagittal cryostat section of bovine epidermis labeled with serum F (1:100 dilution). Note the punctate labeling of cellular boundaries in stratum corneum. The outermost cornified layer was not labeled (*asterisk* in *A*). In *C*, control experiment in which the serum F was preabsorbed with purified lamin B (see Materials and Methods). (*D*) Immunofluorescence picture of a deeper layer (probably stratum granulosum and/or stratum spinosum) in which nuclei as well as cellular boundaries were labeled with serum F (*arrows*). (*E* and *F*) Immunoelectron microscopy of an ultrathin cryosection of muzzle epithelium in which desmosomes labeled with serum F were either observed in tangential (*E*, *arrows*) or in transverse (*F*) sections. In *F*, gold granules are confined to desmosomal plaques (*arrows*) and not to the core of this desmosome. Bars: (*A–D*) 20 μm; (*E*) 1 μm; (*F*) 0.5 μm.
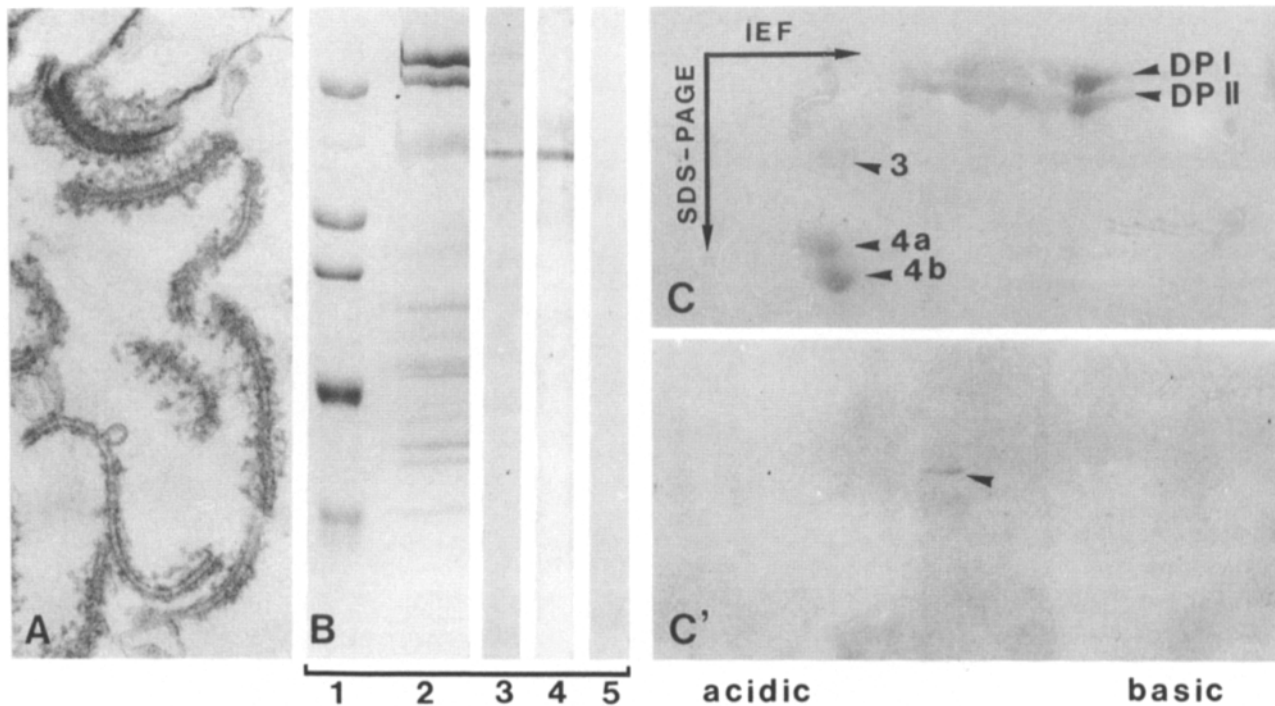
Figure 2. Identification of the immunoreactive component of the desmosome. (*A*) Electron microscopy view of the purified desmosomal sample prepared according to Skerrow and Maltosy (1974). The fraction contained almost exclusively desmosomal regions of the plasma membrane. (*B*) Immunoblotting analysis of the desmosomal proteins separated by one-dimensional PAGE. (Lane *1*) Molecular mass standards: 200, 116, 97, 66, and 42 kD (*top* to *bottom*). (Lane *2*) Coomassie blue staining of desmosomal proteins separated on a 8% SDS-PAGE. (Lane *3*) Immunoblotting analysis of desmosomal proteins with serum F (1:200 dilution). Note the major M_r ~140-kD polypeptide. (Lane *4*) Same analysis using a rabbit anti–lamin B antibody. An identical M_r ~140-kD polypeptide is recognized. (Lane *5*) Control experiment performed with serum F (1:200 dilution) preabsorbed with purified lamin B (see Materials and Methods). Note the disappearance of the 140-kD component. (*C* and *C'*) Western blotting analysis of desmosomal proteins separated by two-dimensional gel. (*C*) Ponceau red staining of the upper part of the blot showing the major high molecular mass components of the desmosomes. DPI/DPII (desmoplakins I and II, M_r ~210-215 kD, IP ~6.8-7.2), band 3 (desmoglein 1:M_r ~160 kD, IP ~4.8), band 4a/4b (desmoglein 2 and 3:M_r ~110-115 kD, IP ~4.8-4.9). (*C'*) Probing of the same part of blot with serum F (1:200 dilution). The arrow points to a single component (M_r ~140 kD; IP ~5.4 + 0.2).

sections mounted in Cityfluor antibleach agent (City University, London, UK) were observed with a Leitz Aristoplan photomicroscope equipped with epifluorescence illumination (N2 filter for rhodamine) and with a Plan Apo 100×, NA 1.32 immersion optics. Photographs were made with Kodak T Max 400 films and developed according to the manufacturer's instructions.

Immunoelectron Microscopy

Electron microscopic immunolocalization was achieved on ultrathin frozen sections and immunogold labeling essentially as described by Miller et al. (1987). Small blocks of fresh bovine muzzle epidermis were fixed in a mixture of 2% paraformaldehyde; 0.05% glutaraldehyde in phosphate buffer pH 7.4 for 2 h at room temperature or overnight at 4°C. Pieces of tissue were then infiltrated in a series of sucrose/PBS (0.5-2.3 M) solution, mounted in specimen holders and rapidly frozen in Freon R22 cooled in liquid nitrogen. Thin (~100 nm) sections were cut with an ultracut automatic microtome (model E; Reichert Science Instruments, Buffalo, NY) equipped with the cryoattachment (optimal temperature settings were −90°C for the knife and −100°C for the specimen). Sections were transferred from the knife to carbon-coated collodion grids with a droplet of cold 2.3 M sucrose and then processed for immunolabeling. Quenching of free aldehyde groups was achieved in PBS-glycine (50 mM), pH 7.4 (5 min) and followed by an incubation with 10% FCS in PBS (30 min). Grids were then incubated with the anti–lamin antibodies (1:100) in PBS containing 10% FCS for 1 h at room temperature, washed (six times for 15 min in PBS) and finally labeled with 10 nm gold conjugated protein A prepared according to Slot and Geuze (1985). After labeling, sections were washed in PBS (four times for 10 min) then in distilled water (three times for 5 min) and stained in neutral uranyl acetate for 10 min. Sections were observed in the microscope (Philips EM 400) without further embedding.

Results

Anti–Lamin B Antibodies Reveal the Desmosomal Plaque in Bovine Stratified Epithelium

Immunofluorescence experiments were carried out on cryostat sections (thickness ~4 μm) of paraformaldehyde-fixed bovine internasal epidermis using previously characterized lamin B antibodies (Guilly et al., 1987).

As shown in Fig. 1, *A–D*, a punctate labeling of the cell boundaries, characteristic of desmosomal labeling, was observed. With the exception of the uppermost cells of stratum corneum, the labeling was associated with cell layers of epithelial cells in stratum corneum, stratum granulosum, and stratum spinosum. As expected, nuclei were also labeled in the nucleated cells of the latter strata (Fig. 1 *D*). The spotty and weak labeling of nuclei may be the result of the degenerative process in which nuclei as well as other organelles are engaged in these cells. Inhibition experiments carried out with anti–lamin B antibodies preabsorbed on purified lamin B (see Materials and Methods) resulted in the total extinction of the fluorescence pattern (see also Fig. 2) of both nuclei and cell borders (Fig. 1 *C*). A similar pattern of labeling was obtained with polyclonal antibodies raised against *Torpedo* lamin B (not shown).
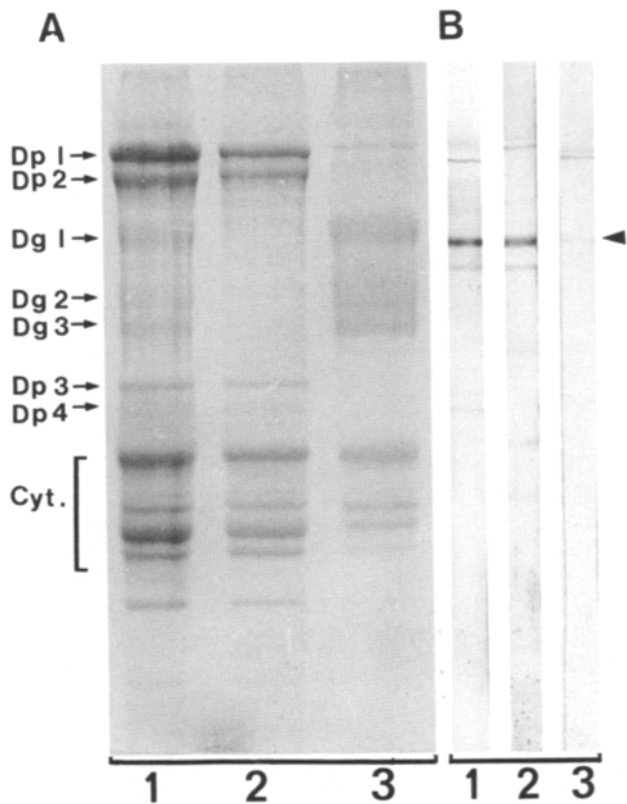
with pure lamin B obtained from rat liver (see Fig. 2 *B*, lane *5*). Interestingly, the M_r ~140-kD band was also recognized by polyclonal antibodies raised against lamin B purified from *Torpedo* liver nuclear envelopes (Fig. 2 *B*, lane *4*). No immunoreactivity was detected with antibodies directed against lamin A and C (not shown) thus confirming that the immunoreactive epitopes of the M_r ~140-kD protein are specific for lamins of the B type. The 140-kD polypeptide represents a quantitatively minor component of the desmosomal fraction since it is not detected by Coomassie blue staining (cf. Fig. 2 *B*, lane *2*). Thus, the 140-kD protein does not likely correspond to any previously identified desmosomal protein. Indeed, two-dimensional immunoblotting experiments (Fig. 2, *C* and *C'*) readily excluded the possibility of a cross-reaction with desmoglein 1 (dg1) or band 3 (M_r ~160–175 kD) (Mueller and Franke, 1983; Cowin et al., 1984) since their (IPs) are different. The 140-kD polypeptide possesses an acidic IP of ~5.4; this acidic IP being characteristic of lamins of type B.

Figure 3. Distribution of the 140-kD protein in desmosomes dissected into cytoplasmic protein and membrane glycoprotein domains. (*A*) Coomassie blue staining of desmosomal fractions separated on 8% SDS-PAGE (5 µl of each sample were loaded in the wells). (Lane *1*) Untreated desmosomes; (lane *2*) urea extract; (lane *3*) residual urea pellet. Dp 1-4 predominated in 9 M urea extract while Dg 1-3 were present in the residual pellet. *Cyt*, cytokeratins. (*B*) Immunoblotting detection of the 140-kD protein (serum F 1:200) after SDS-PAGE. (Conditions are similar to *A*). (Lane *1*) Untreated desmosomes; (lane *2*) urea extract; (lane *3*) residual urea pellet. Lamin B immunoreactivity (*arrow*) was associated with the urea soluble extract.

Further characterization of these membrane domains was achieved by immunogold labeling of ultrathin frozen sections of bovine epidermis. Desmosomes, either viewed in tangential (Fig. 1 *E*) or in transverse sections (Fig. 1 *F*), were labeled. In transverse sections, which allow the visualization of the different desmosomal regions (Miller et al., 1987), gold granules were observed in association with desmosomal plaques and not with the extracellular space (desmosome cores).

Identification of the Immunoreactive Component(s) of the Desmosome

By using immunoblot analysis of SDS-PAGE separated polypeptides from purified desmosomal preparation (Fig. 2), we have identified a polypeptide band with a relative molecular mass of ~140 kD, which cross-reacted specifically with the anti–lamin B antibodies (serum F). In some experiments, a second band with a lower molecular mass was also revealed (Fig. 2 *B*, lane *3*). Specificity of the immunoreactivity was ensured through the complete inhibition of the immunodetection of the 140-kD band after preabsorption of serum F
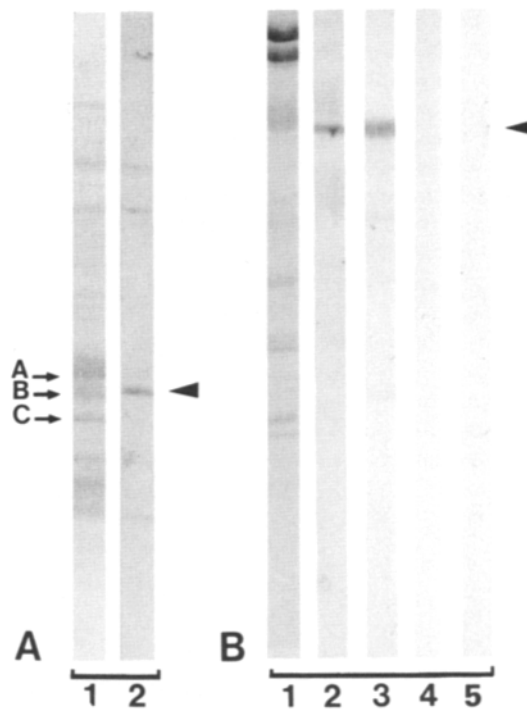

Figure 4. Detection of vimentin binding on desmosomal polypeptides by an overlay assay. The ability of rat liver nuclear envelopes (*A*) and desmosomal proteins (*B*) to bind vimentin after electrophoretic transfer to nitrocellulose was detected by incubation with vimentin then with anti–vimentin antibody. (*A*) Lane *1*, Coomassie blue staining of rat liver nuclear envelopes proteins separated on a 10% SDS-PAGE run for an extra 15 min to improve the separation of the three lamins; lane *2*, binding assay with vimentin revealed almost exclusively lamin B. (*B*) Lane *1*, Coomassie blue staining of desmosomal proteins separated on a 8% SDS-PAGE; lane *2*, immunoblotting using rabbit anti–lamin B antibodies showing the position of the ~140-kD protein; lane *3*, vimentin binding assay (note the strong binding of vimentin to the 140-kD protein); lane *4*, control experiment in which vimentin was omitted and showing that no component of the desmosome was revealed with anti–vimentin antibody alone; lane *5*, inhibition of the binding of vimentin to the M_r ~140 kD was achieved after preincubation of the strip with anti–lamin B antibodies (1:100) before incubation with vimentin.

To further investigate to which desmosomal compartment the 140-kD protein was associated, we have dissected the desmosomes into cytoplasmic (peripheral), plaque proteins, and intrinsic membrane glycoproteins according to the method of Skerrow et al. (1987). In urea extraction experiments (9 M urea, pH 7.5) we showed (Fig. 3 A) a clear separation of plaque proteins (desmoplakins [Dp] 1-4; soluble in urea), from most core glycoproteins (desmogleins [Dg] 1-3; recovered in the pellet). Immunoblotting experiments demonstrated that the immunoreactivity associated with the 140-kD protein was mostly present in the soluble urea extract (Fig. 3 B, lane 2). This demonstrated that the 140-kD protein is a peripheral plaque protein.

The M_r ~140-kD Protein of the Desmosome Binds IF Proteins In Vitro

An expected feature for the presence of lamin B related epitopes in the 140-kD protein would be its ability to bind IF proteins. We addressed this issue by way of an in vitro assay in which vimentin binding was tested on desmosomal components transferred onto nitrocellulose paper after separation by one-dimensional SDS-PAGE. The validity of the in vitro binding assay was first verified with a preparation of purified rat nuclear envelopes. Fig. 4 A shows that lamin B was the main vimentin binding component (Fig. 4 A, lane 2) as previously demonstrated by Georgatos et al. (1987). Under the same experimental conditions, the M_r ~140-kD protein of the desmosome (Fig. 4 B, lane 2) also bound vimentin (Fig. 4 B, lane 3). Occasionally, another faint vimentin binding was observed in the 60-65-kD region of the blot probably corresponding to the cytokeratins. However, vimentin binding to other desmosomal components as well as binding of unrelated cytoskeletal proteins such as α or β tubulin were not detected in these experiments (not shown). Vimentin binding on the M_r ~140-kD protein was totally abolished after preincubation of nitrocellulose strip with anti–lamin B antibodies before incubation with vimentin (Fig. 4 B, lane 5).

Discussion

Desmosomes are prominent plasma membrane domains where bundles of intermediate filaments are anchored (Drochmans et al., 1978; Denk et al., 1985). In rat mammary cells, they are also engaged in the nucleation and organization of IF bundles (Bologna et al., 1986). This suggests that desmosomes exhibit unique IF binding properties compared with the nondesmosomal plasma membrane.

In this work, we show that a previously unrecognized protein of the desmosome from bovine epithelium shares common epitope(s) and thus structural domains with lamin B, and that it is engaged in IF binding in vitro. This desmosomal protein has a M_r of ~140 kD with an electric point of ~5.4. The cytological and ultrastructural localization as well as biochemical characterization of this protein were performed using polyclonal antibodies to lamin B of either autoimmune or postimmunization origin. In all experiments, both lamin B antibodies gave similar results.

The 140-kD polypeptide is a minor component since it is not detected by Coomassie blue staining of gels of purified desmosomal fractions. It does not correspond to any previously identified component of the desmosomes (reviewed in

Franke et al., 1987) and, in particular, to desmogleins, the major glycoproteins of the desmosome. In two-dimensional gel electrophoresis, the 140-kD protein is readily separated from Dg1 (band 3) and Dg2 (bands 4a, 4b), which are more acidic. Moreover, the association of the 140-kD protein with desmosomal plaques as demonstrated by immunogold labeling and urea extraction experiments readily distinguishes it from core proteins. Since this protein is localized at the cytoplasmic side of the membrane at site of IF/membrane contacts (i.e., the desmosomal plaque), it may be involved in the attachment of IF to desmosomes. Taken together, our data suggest that a domain(s) of functional significance for IF binding is shared by lamin B and the 140-kD protein from the desmosome.

A property that can be expected from a lamin B–related protein is its ability to bind IF proteins. In an in vitro binding assay, we observed that the 140-kD component is the only desmosomal component able to bind detectable amounts of vimentin. As initially reported for authentic lamin B (Georgatos and Blobel, 1987b), an inhibition of vimentin binding by lamin B antibodies was also observed in our experiments. It may be argued that the vimentin binding to desmosomal protein is not relevant to the association of cytokeratins with desmosomes in stratified epithelia. However, in myocardium, Purkinje fiber cells (Kartenbeck et al., 1983; Thornell et al., 1985), arachnoidal meningional cells, meningiomas, and in some other cultured epithelium-derived cells (Kartenbeck et al., 1984; Cowin et al., 1985; Moll et al., 1986), the association of desmosomes with desmin or vimentin IF has been observed.

There is currently no consensus on the molecular component(s) that may be responsible for IF binding at the desmosomal plaque. Nonetheless, several components of the desmosome have been tentatively assigned as potential cytokeratin binding proteins. Tsukita and Tsukita (1985) described a 240-kD protein (desmocalmin) of the desmosomal plaque that binds calmodulin in vitro in a calcium dependent manner. This protein also binds to keratin filaments in vitro. However, this protein is absent from desmosomes in the stratum corneum. On the other hand, a 75-kD desmosomal protein (Dp 4) was also reported to bind type I cytokeratins (Kapprell et al., 1988). Since this protein is not distributed in all desmosomes, it cannot function in IF binding in general. The heterogeneous distribution of these putative IF binding proteins may be correlated to either differences in the composition of desmosomes and cytokeratins in different epithelia, and/or to the differentiation stage of the cells in a given epithelium (Moll et al., 1982; Eichner et al., 1984; Sun et al., 1984; Skerrow et al., 1989; Steinberg et al., 1987). Thus, the various putative IF binding proteins of the desmosome would provide optimized binding sites for IF anchoring according to the tissue or to the differentiation stage of the cells. In bovine muzzle epithelium, the lamin B–related 140-kD protein is represented in the stratum corneum, stratum granulosum, and stratum spinosum. It is remarkable that the 140-kD component is prominent in the nuclei-free cells of the stratum corneum, which is not the case for desmoplakins. This may be relevant to the peculiar IF organization in these highly differentiated cells (Farquhar and Palade, 1965).

In the model proposed by Georgatos and Blobel (1987a), the structurally apolar intermediate filaments acquire polar

features through their different nucleation and capping sites. In epithelial tissue, the observation that desmosomes may represent nucleation sites for the carboxy termini of intermediate filament suggests that receptors for the NH_2 termini may exist in other cell compartment(s). Such a putative receptor may be ankyrin, the main vimentin capping site at the plasma membrane (Georgatos and Marchesi, 1985). Our attempts to detect ankyrin in bovine epithelium by immunofluorescence were unsuccessful, thus, if cytokeratin filaments behave as vimentin ones, their other putative capping site remain to be identified.

In conclusion, we have identified a 140-kD acidic protein of the desmosomal plaque of bovine muzzle epithelium that shares epitope(s) with lamin B. This protein also binds vimentin in vitro. As suggested for another lamin B–related protein, the 54-kD protein from *Torpedo* electrocyte and rat neuromuscular junction (Cartaud et al., 1989; Cartaud, A., J. C. Courvalin, B. Jasmin, and J. Cartaud. 1989. *J. Cell Biol.* 104[4, Pt. 2]:133a[Abstr.]), this peripheral plasma membrane protein may also be involved in the nucleation of IF at the plasma membrane. A major implication of our data is that, in addition to nuclear lamin B, a family of lamin B–related proteins may provide the cells with several nucleation sites, which may account for the diversity of IF organization in tissues.

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