

Plakophilins 2a and 2b: Constitutive Proteins of Dual Location in the Karyoplasm and the Desmosomal Plaque

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Abstract. Using antibodies and recombinant DNA techniques, we have identified plakophilin 2, a novel desmosomal plaque protein of M_r 100,000 (estimated from SDS-PAGE), which is a member of the *arm*-repeat family of proteins and can occur in two splice forms (2a and 2b) because of the insertion of a 44 amino acid (aa)-encoding exon. In its aa sequence (837 and 881 aa, calculated pIs: 9.33 and 9.38, mol wts 92,750 and 97,410 kD), it is conspicuously related to the 80-kD plakophilin 1, with which it shares a central region of 9 repeats of the *arm*-motif, preceded by a long head region and followed by a very short (11 aa) carboxy-terminal sequence. Plakophilin 2 and its mRNA have been detected in a wide range of tissues and cell types, including cells devoid of desmosomes. By light and electron microscopical immunolocalization, plakophilin 2 has been localized to plaques of desmosomes of one-

layered ("simple") and complex epithelia, carcinomas, diverse epithelium-derived cell culture lines, as well as cardiac tissue and the dendritic reticulum cells of lymphatic germinal centers, i.e., desmosomes in which plakophilin 1 is not detected. However, plakophilin 2 has also been localized in the desmosomes of certain but not all stratified epithelia where it coexists with plakophilin 1. Remarkably, plakophilin 2 is also enriched in the karyoplasm of a wide range of cell types, including many that lack desmosomes and in which, therefore, the nuclear state is the only locally enriched form of plakophilin 2 present. We conclude that plakophilins 2a and 2b are basic nuclear proteins that in certain cell types additionally assemble with other proteins to form the desmosomal plaque and serve general nuclear functions as well as a function specific to many but not all desmosomes.

AMONG the diverse forms of plaque-coated adhering junctions (12; for reviews see references 66, 72), the desmosomes are a particularly well-defined category: They represent clusters of certain isoforms of two types of transmembrane glycoproteins, the desmogleins (Dsg1-3) and desmocollins (Dsc1-3), members of the larger cadherin family of cell adhesion molecules, which at their carboxy-terminal cytoplasmic portions assemble the common plaque protein, plakoglobin, and several desmosome-specific proteins. These include general desmosome components such as desmoplakin I and cell type-specific proteins such as desmoplakin II and plakophilin 1 (originally identified as "band-6-protein" of desmosomal fractions from bovine muzzle; for references see 10, 21, 26, 32, 68). One of the desmosomal plaque proteins, plakoglobin, which has also been shown to occur in non-desmosomal adhering junctions (8, 17, 18) and in a soluble cytoplasmic pool (30), has been identified, on the basis of

its amino acid (aa)¹ sequence (13, 19), as a member of a group of proteins characterized by variable numbers of repeats of a motif of ~42 aa, the so-called *arm*-repeats first identified in the developmentally defined gene *armadillo* of *Drosophila* (58; for references see 57, 59, 66). This *arm*-repeat protein family also includes β -catenin, a plaque protein specific for nondesmosomal adherens junctions (e.g., references 46, 51, 54), and the cell type-specific, i.e., "accessory" (cf. reference 68) desmosomal plaque protein, plakophilin 1 (63). The aa sequence of plakophilin 1 (80,496 kD; pI: 9.34) shows that it is a sort of "naturally truncated" *arm*-family protein, as it contains only nine *arm*-repeats, followed by a very short (13 aa) carboxy terminal segment (25, 26, 66). As we have not been able to detect plakophilin 1 in desmosomes of a wide range of cell types (26, 31, 66) but have noticed cytoskeletal proteins immunologically cross-reactive with plakophilin 1 (26), we have systematically examined the possible existence of dif-

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1. *Abbreviations used in this paper:* aa amino acid; NFD, nonfat dry milk powder; NLS, nuclear localization signal; PBST, PBS containing 0.02% Tween; PLC, primary liver carcinoma; TBST, TBS containing 0.1% Tween.

ferent plakophilin 1-related proteins that may be synthesized in single-layered ("simple") epithelia and in nonepithelial, desmosome-possessing tissues such as myocardium, i.e., cells without desmosomal plakophilin 1 reaction. Here we report the discovery of plakophilin 2, which can occur in two isoforms and is a widespread desmosomal plaque constituent not only in simple epithelia and certain nonepithelial tissues such as myocardium and dendritic reticulum of lymph node follicles, but also in certain stratified epithelia. We also show that the gene encoding plakophilin 2 is also expressed in a variety of cell types devoid of desmosomes and that it is, in all these cells, highly enriched in the karyoplasm.

Materials and Methods

Tissues and Cultured Cells

Specimens of human duodenum, liver, stomach, pancreas, vagina, esophagus, tongue, salivary gland, bladder, epidermis, heart, lymph nodes, colon, and mammary gland carcinomas and several other tumors were snap-frozen in isopentane cooled by liquid nitrogen to about -140°C (e.g., references 49, 64, 65). Samples of bovine tissues were obtained freshly from a local slaughterhouse (e.g., references 14, 15), samples of rat tissues were obtained from the animal facility of this research center, and both were frozen as mentioned above.

Cell cultures used in this study included the following human lines: colon adenocarcinoma Caco-2 and HT29, primary liver carcinoma (PLC), mammary gland carcinoma MCF-7, vulvar squamous carcinoma-derived A-431, keratinocytes HaCaT, SV-40-transformed (SV-80) fibroblasts, glioma U 333 CG/343 MG, erythroleukemia K562, and endothelial ECV 304. In addition, we examined the following animal cell lines: bovine (calf) lens-forming cells, bovine mammary gland BMGE+H C15, bovine kidney MDBK, bovine CPAE endothelial cells, canine MDCK, rat neuroendocrine PC12, rat vascular smooth muscle-derived RVF cells, mouse 3T3-L1 preadipocytes, and rat kangaroo PtK₂ (for sources of cell lines, see American Type Culture Collection, Rockville, MD, and previous reports from this laboratory: references 2, 17, 18, 36, 64). Cells were maintained under standard conditions and examined at different cell densities, from freshly seeded (24 h) at low density to preconfluence and confluence.

Antibodies and Antibody Production

Guinea pig antibodies specific for plakophilin 2 were obtained by immunization with synthesized peptides (67) representing various parts of the aa sequence deduced from cDNA sequencing. All results shown in this report have been obtained with antibodies against the aa sequence (one-letter code) VKEQYQDVPMPPEEKs, representing a segment at the border between *arm*-repeats 5 and 6, which were used after affinity-purification on the iodoacetyl-immobilized peptide: 5 mg peptide were conjugated to 1 ml iodoacetyl-coupled gel matrix (UltraLink™ Iodoacetyl; Pierce, Rockford, IL) as described in the manufacturer's instructions. The affinity matrix (1 ml bed volume) was equilibrated with PBS (140 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, pH 7.4) containing 0.02% Tween (PBST) and incubated with the eluted antiserum (1:3 in PBST) for 1 h at room temperature under shaking. After a column wash with 10 bed volumes PBST, the specifically bound antibodies were eluted with 100 mM glycine at pH 2.8.

Mouse mAbs to plakophilin 2 were generated using the procedure described (65) by immunization with synthesized peptides representing the carboxy terminal aa sequence KKAQFKKTDVNSRTAKAYHSLKLD. The resultant hybridoma cell culture supernatants were screened by immunofluorescence microscopy on cryostat sections of human heart and by immunoblotting of total cellular proteins of human PLC cells separated by SDS-PAGE. From a total of 11 different hybridomas, 8 mAbs (PP2-62, PP2-77, PP2-86, PP2-125, PP2-150, PP2-218, PP2-233, and PP2-320) were characterized in detail and routinely used.

For comparison with plakophilin 2 antibodies, various murine mAbs against desmoplakins (7), plakoglobin (PG5.1, reference 7; PG11E4, kindly provided by Dr. M. Wheelock, University of Toledo, OH), plakophilin 1 (5C2; reference 26; antibodies available from Progen Biotechnik, Heidelberg, Germany), vinculin (11/5; Bio-Yeda, Rehovot, Israel), and

β -catenin (Dianova, Hamburg, Germany) were used. Rabbit and guinea pig antibodies to desmoplakins were obtained as described (e.g., reference 15), and rabbit antibodies to E-cadherin, α -catenin or β -catenin were generously provided by Dr. R. Kemler (Max Planck Institute for Immunology, Freiburg i. Br., Germany).

Secondary antibodies used for immunofluorescence microscopy were Cy3-, Cy2-, and FITC-conjugated goat antibodies to immunoglobulins of guinea pig, mouse, or rabbit (Dianova; Cy2-conjugated antibodies from BioTrend, Cologne, Germany), and those used for immunoelectron microscopy were anti-guinea pig IgG coupled to 1- or 5-nm colloidal gold particles (BioTrend). Immunoblotting experiments were performed with HRP-conjugated antibodies to guinea pig or mouse immunoglobulins (Dianova).

cDNA Library Screening and DNA Sequencing

Approximately 1×10^6 plaques of a λ -ZAP II cDNA expression library representing human heart (atrium), generously provided by the "Genzentrum München," were screened using a 230-bp PCR fragment generated with specific oligonucleotides representing a plakophilin 1-like 412-bp expressed sequence. A single positive 2,321-bp cDNA clone (pp2-3-2.3) was isolated, and exonuclease deletion fragments were prepared using a "nested deletion" kit (MBI Fermentas, Frankfurt, Germany), subcloned into pBluescript II (SK-) (Stratagene, Heidelberg, Germany), and sequenced. A 950-bp fragment corresponding to the 5'-end of clone pp2-3-2.3 was then used to generate randomly primed probes for screening a second λ -ZAP II human heart library (Stratagene) from which 11 overlapping partial cDNA clones with lengths up to 1.6 kb were isolated, including a 1.2-kb clone (pp2-4-1.2) containing an additional 300-bp amino terminal segment that was used for rescreening the same library. The isolated 1-kb cDNA clone (pp2-5-1.0) presented a further amino terminal extension of 300 bp, and the most 5'-located 600-bp fragment was used to screen a λ gt11 human colon carcinoma cell (line CaCo2) cDNA expression library (cf. reference 19) from which a 3212-bp cDNA clone (pp2-1-3.2) was isolated. This clone was subcloned and completely sequenced. Sequence alignment revealed a partial 1.4-kb overlap with clone pp2-2-2.3, a full overlap with clone pp2-3-1.0 except for a 132-bp insertion, and an additional 1.2-kb amino terminal fragment.

Using the first 1,000 nucleotides of clone pp2-1-3.2 for rescreening the same library, a 2,812-bp cDNA clone was isolated (pp2-2-2.8) that turned out to be a full-length clone comprising a modified initiation sequence before the start codon.

Northern Blot Analysis, Transcription, and Translation In Vitro

Total or poly (A)⁺ RNA from diverse cultured human cell lines and total RNA from various normal and malignant human tissues were isolated essentially as described (64, 65). Total RNA from human heart was also obtained from Clontech Labs (Palo Alto, CA). In Northern blot analyses, ³²P-labeled antisense riboprobes derived from clone pp2-1-3.2 were used (for details see reference 64).

Clone pp2-2-2.8 was transcribed and translated in vitro in the presence of [³⁵S]methionine in a rabbit reticulocyte lysate system (Promega, Heidelberg Germany) according to the manufacturer's instructions. Products were separated by SDS-PAGE, stained with Coomassie blue, destained, soaked for 15 min in Amplify solution (Amersham Buchler GmbH, Braunschweig, Germany), dried, and exposed to X-ray film (Eastman kodak, Rochester, NY) at -80°C .

Cell Fractionation

Cytoskeletal fractions of cultured cells were prepared according to Achstatter et al. (1) or following a modification of the protocol of Herrmann and Wiche (27). Cells attached to 10-cm-diam culture dishes were washed three times with PBS and extracted with 1 ml lysis low buffer (0.5 \times PBS, 50 mM Mops, pH 7.0, 10 mM MgCl₂, 1 mM EGTA, 0.2% NP-40 [wt/vol], 2 mM PMSF) at room temperature for 2 min with gentle agitation. Proteins released were recovered in the supernatant after 15,000 g centrifugation. Detergent-resistant cytoskeletal residues were solubilized from culture dishes by incubation with 1 ml lysis high buffer (i.e., lysis low buffer without EGTA, containing 1% NP-40, complemented with 50 μ g DNase I and 10 μ g RNase A/ml) on ice for 3 min and then by agitation, i.e., sucking up and down in an automatic pipette. Then 0.2 ml 5 M NaCl was added to 0.8 M final concentration and the active resuspension was repeated for 3 min.

Cytoskeletal residues were finally collected by centrifugation at 15,000 g for 15 min at 4°C.

Alternatively, cells were initially permeabilized in lysis low buffer containing digitonin (0.005% wt/vol) instead of NP-40, and residual material was collected by scraping off the dish with a rubber policeman, followed by centrifugation at 15,000 g for 15 min. The supernatant was removed after taking an aliquot, and the pellet was resuspended in 1 ml lysis low buffer containing 0.1% NP-40, followed by recentrifugation as mentioned above. Again, an aliquot sample of the supernatant was taken and the pellet material further extracted in lysis buffer containing 1% NP-40. After another centrifugation, both the final pellet and the supernatant were analyzed.

For more detailed characterization of the soluble plakophilin 2, 100,000-g supernatant fractions were prepared as follows: 1 ml Triton X-100 buffer (10 mM Tris-HCl, pH 7.4, 3 mM MgCl₂, 0.15% Triton X-100, 1 mM DTT, 50 µg pepstatin, and 50 µg leupeptin/ml) was added per PBS-washed culture dish, and cells were collected with a rubber policeman and manually homogenized in a Dounce glass homogenizer by 20 strokes (1 µl benzonase/ml suspension was added). The cell homogenate was centrifuged at 15,000 g for 20 min, and the obtained supernatant was then further centrifuged at 100,000 g for 2 h at 4°C. In parallel, "total cell homogenates" from one 10-cm culture dish were precipitated with methanol.

Gel Electrophoresis and Immunoblotting

Procedures for SDS-PAGE in 8 or 10% acrylamide gels and two-dimensional gel electrophoresis have been described (1). For immunoblotting, the polypeptides separated were electrophoretically transferred to nitrocellulose, and the membranes blocked by incubation in 5% nonfat dry milk powder (NFDM) in TBS with 0.1% Tween (TBST) for 1 h. Blots were incubated for 1 h with primary antibodies diluted to appropriate concentrations, washed in TBST, and incubated for 1 h with HRP-conjugated antibodies to guinea pig or mouse immunoglobulins (diluted 1:10,000 in 5% NFDM in TBST). After washing again in TBST, the antibodies bound were visualized with the ECL reagent (Amersham Buchler GmbH).

For controls, nitrocellulose blot sheets reacted with guinea pig antibodies against plakophilin 2 were washed with TBST for 30 min, reblocked with NFDM, and then re probed with murine mAb PP1-5C2 against plakophilin 1 (26) as specified above, using again ECL for detection.

Immunofluorescence Microscopy

Immunofluorescence microscopy of sections through frozen tissues and of monolayers of cultured cells was performed by using several procedures optimized for the detection of soluble antigens (see also references 3, 34, 40, 41). For immunohistochemistry of cell cultures, cells grown on polylysine-coated coverslips were washed briefly in PBS containing 2 mM MgCl₂ and fixed at -20°C in methanol (5 min) and acetone (30 s). Frozen tissue sections were mounted on poly-lysine coated coverslips, air-dried, and then fixed for 10 min either in -20°C acetone or in 2% formaldehyde in PBS, freshly made from paraformaldehyde, followed by a rinse in water, 5 min quenching in 50 mM NH₄Cl in PBS, and another PBS wash.

Cell and tissue specimens were briefly air dried before incubation with the antibodies according to one of the following protocols: (1) Specimens were incubated in PBS containing 0.2% Triton X-100 for 5 min and then washed for a further 5 min, before application of antibodies as in protocol 3. (2) Specimens were incubated in PBS containing 0.1% saponin for 5 min, followed by two 2-min PBS washes. (3) Cells or tissues were rinsed in PBS, and primary and secondary antibodies were applied for 20 or 30 min in a humid chamber, with three 2-min or 5-min PBS washes between each incubation. After the binding of secondary antibodies, specimens were washed again in PBS, rinsed in water, dipped in ethanol, dried, and mounted. Immunofluorescence was documented with a photomicroscope (Axiophot; Carl Zeiss, Jena, Germany).

Confocal laser-scanning immunofluorescence microscopy was done on a Zeiss LSM 410 UV (Carl Zeiss). For simultaneous double-label fluorescence, an Argon ion laser operating at 488 nm and a Helium-neon laser operating at 543 nm were used together with a band-pass filter combination of 510-525 and 590-610 for visualization of Cy-2 or FITC and Cy-3 fluorescence.

Immunoelectron Microscopy

Frozen tissue sections (2 or 5 µm) were fixed in acetone at -20°C for 10 min and treated with 0.5% Triton X-100 for 5 or 10 min. Cells grown on

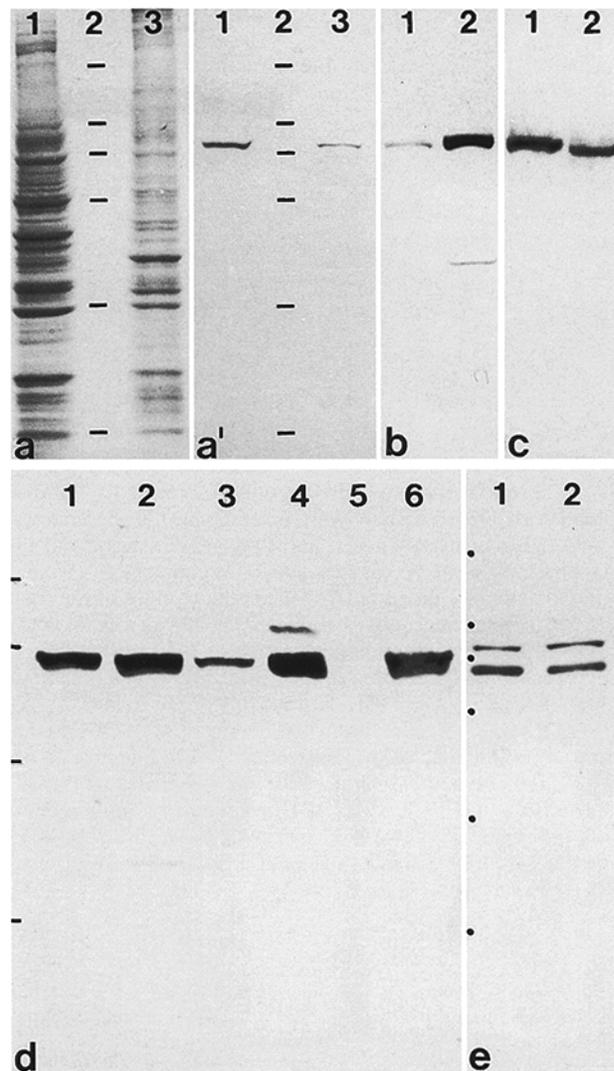


Figure 1. Identification of plakophilin 2 in cellular fractions of various human cell culture lines by SDS-PAGE (a-c, e, 10%; d, 8%) and immunoblotting using purified guinea pig antibodies. (a) Coomassie brilliant blue-stained proteins of the NP-40-soluble (lane 1) and the cytoskeletal (lane 3) fractions of HT29 colon carcinoma cells. (a') Radiogram corresponding to a, showing immunochemiluminescence detection of plakophilin 2 at a 100K mol wt position. Reference proteins (lane 2) are, from top to bottom, of molecular weights 200K, 116K, 97K, 66K, 45K and 29K. (b) Immunoblot (as in a') of total cellular proteins from PLC hepatocellular (lane 1) and Caco-2 colon (lane 2) carcinoma cell cultures. (c) Immunoblot of proteins of the NP-40-insoluble cytoskeletal fraction (lane 1) and of total cell homogenate (lane 2) of A-431 vulvar carcinoma cells. (d) Immunoblotting reaction of plakophilin 2 on total cellular proteins from diverse epithelial and nonepithelial cell lines: PLC (lane 1), MCF-7 breast carcinoma cells (lane 2), SV-40-transformed fibroblasts SV-80 (lane 3), glioma U333CG/343 (lane 4), blank (lane 5), and endothelial ECV304 (lane 6); reference proteins (bars) were of molecular weights 116K, 97K, 66K, and 45K, respectively. (e) Immunoblot of polypeptides from SV-80 fibroblasts using slightly different SDS-PAGE conditions showing the total NP-40-insoluble cytoskeletal fraction (lane 1) and the corresponding high salt supernatant (lane 2). Note that under the separation conditions chosen a twin band is detected. Reference proteins (dots) as in a.

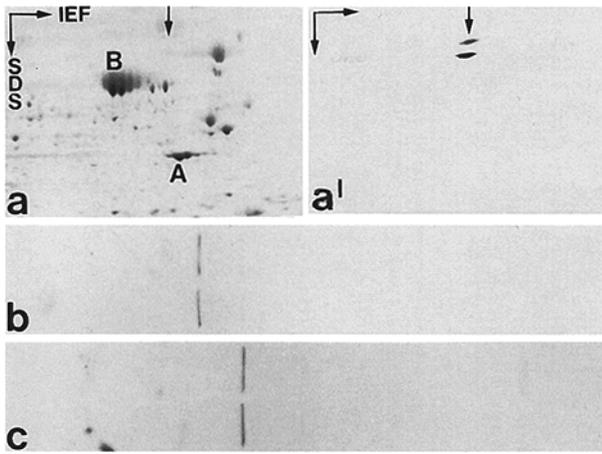


Figure 2. Two-dimensional gel electrophoresis (*a* and *a'*) of plakophilin 2, separated by IEF (*horizontal arrows*) in the first and SDS-PAGE (*downward arrows*) in the second dimension and absence of immunological cross-reaction with plakophilin 1 (*b* and *c*). (*a*) Coomassie brilliant blue-stained polypeptides of the total cell homogenate from cultured human SV-80 fibroblasts. *B*, BSA; *A*, α -actin. (*a'*) Corresponding immunoblot reaction, visualized by ECL, showing the position of the "twin variants" of plakophilin 2 at 100K (*arrow*) and 95K, respectively. (*b*) Two lanes showing SDS-PAGE (direction from left to right) of total proteins from cultured human epidermal keratinocytes of line HaCaT after immunoblot reaction with the antibodies also used in *a'*, visualized by ECL. (*c*) ECL result of reprobing of the immunoblot shown in *b* with the plakophilin 1-specific mAb PP1-5C2. Note that the plakophilin 1 band appears at a different position than the plakophilin 2 band in *b*.

coverslips were fixed with 2% formaldehyde in PBS for 5 min and then permeabilized with 0.1% saponin in PBS for 2 min. Incubation with primary antibodies was usually for 3 h.

As secondary antibodies, anti-guinea pig IgGs coupled to 1- or 5-nm colloidal gold particles ("nanogold") were applied overnight, with or without silver enhancement for 3 min (60). For double label immunolabeling, mAbs against plakophilin 1 (cf. reference 26) or desmoplakins I and II (cf. references 8, 75-77) were applied and detected by reaction with secondary antimurine Ig antibodies coupled to gold particles of 5- or 10-nm diam. Secondary fixation with glutaraldehyde, dehydration, and embedding have been described (60). Electron micrographs were taken with an electron microscope (EM 910; Carl Zeiss, Oberkochen, Germany).

Results

Discovery of Plakophilin 2 by Immunoblot

In our searches for proteins with homology or immunological cross-reactivity to known desmosomal plaque proteins (26), we noted a fragment of an RNA encoding a polypeptide with about 40% sequence identity to the *arm* repeat domain of human plakophilin 1 (see also below). Synthetic peptides representing different segments of this region were used to raise guinea pig antibodies that recognized this new protein but not the related plakophilin 1. Using such antibodies for immunoblotting of SDS-PAGE-separated proteins, we detected, above the plakophilin 1 position at ~ 80 K, an approximately 100K-mol wt polypeptide band, sometimes appearing as a twin band, in a wide range of cultured human cell lines (Fig. 1, *a-e*; see also Fig. 2, *a* and *a'*). These included representatives of simple and

stratified epithelia as well as tumors and cell culture lines such as colon adenocarcinoma HT29 and Caco-2 and liver carcinoma PLC cells (Fig. 1, *a'*, *b* and *d*), mammary gland carcinoma MCF-7 cells (Fig. 1 *d*), A-431 vulvar carcinoma cells (Fig. 1 *c*), and HaCaT keratinocytes (Fig. 2 *b*). This immunoreactive band was also observed in immunoblot analyses of proteins from human heart and colon tissues and several human and bovine stratified epithelia (not shown). Because of its close sequence relationship, we classified the polypeptide as "plakophilin 2." However, cross-reaction between plakophilins 1 and 2 was not observed with the various guinea pig antisera, nor with the mAbs to plakophilin 2 prepared as described in Materials and Methods (see also Fig. 2, *b* and *c*). Unexpectedly, this protein was also detected in immunoblots of total cell homogenates of diverse nonepithelial cells such as SV-80 fibroblasts, erythroleukemia K562, and U333 CG/343 glioma cells (Fig. 1 *d*).

In cell fractionation experiments, the bulk of this reactive protein was recovered in the cytoskeletal fraction (Fig. 1 *c*, lane 1). Depending on the cell type and the homogenization conditions, however, a significant proportion of the protein was often also detected in the detergent-soluble fraction (Fig. 1 *a'*, lane 1). Most of the soluble protein was already released after permeabilization with

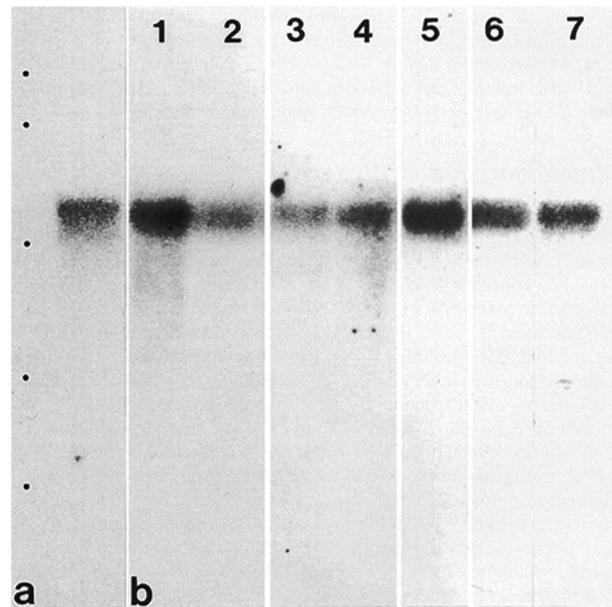


Figure 3. Northern blot analysis of plakophilin 2 in diverse cultured human cell lines, tissues, and carcinomas by using cDNA clone pp2-1-3.2, showing the detection of an approximately 5.3-kb band in (*a*) poly(A)⁺-RNA from CaCo2 human colon carcinoma cell culture (2 μ g; 3 h-exposure) and (*b*) total RNAs (20 μ g/lane) from salivary gland (lane 1), cultured erythroleukemia cells of line K-562 (lane 2), bladder carcinoma (lane 3), pheochromocytoma (lane 4), endometrial carcinoma (lane 5), heart (lane 6), and cultured human glioma cells (lane 7). Signals were detected after RNase digestion after 14- (lane 1-5) and 4-d (lanes 6 and 7) exposure. Note strong intensity variations between the different tissues and cell lines. The positions of the reference RNAs are indicated by dots at the left margin (from top: 9.49, 7.46, 4.4, 2.37, and 1.35 kb).

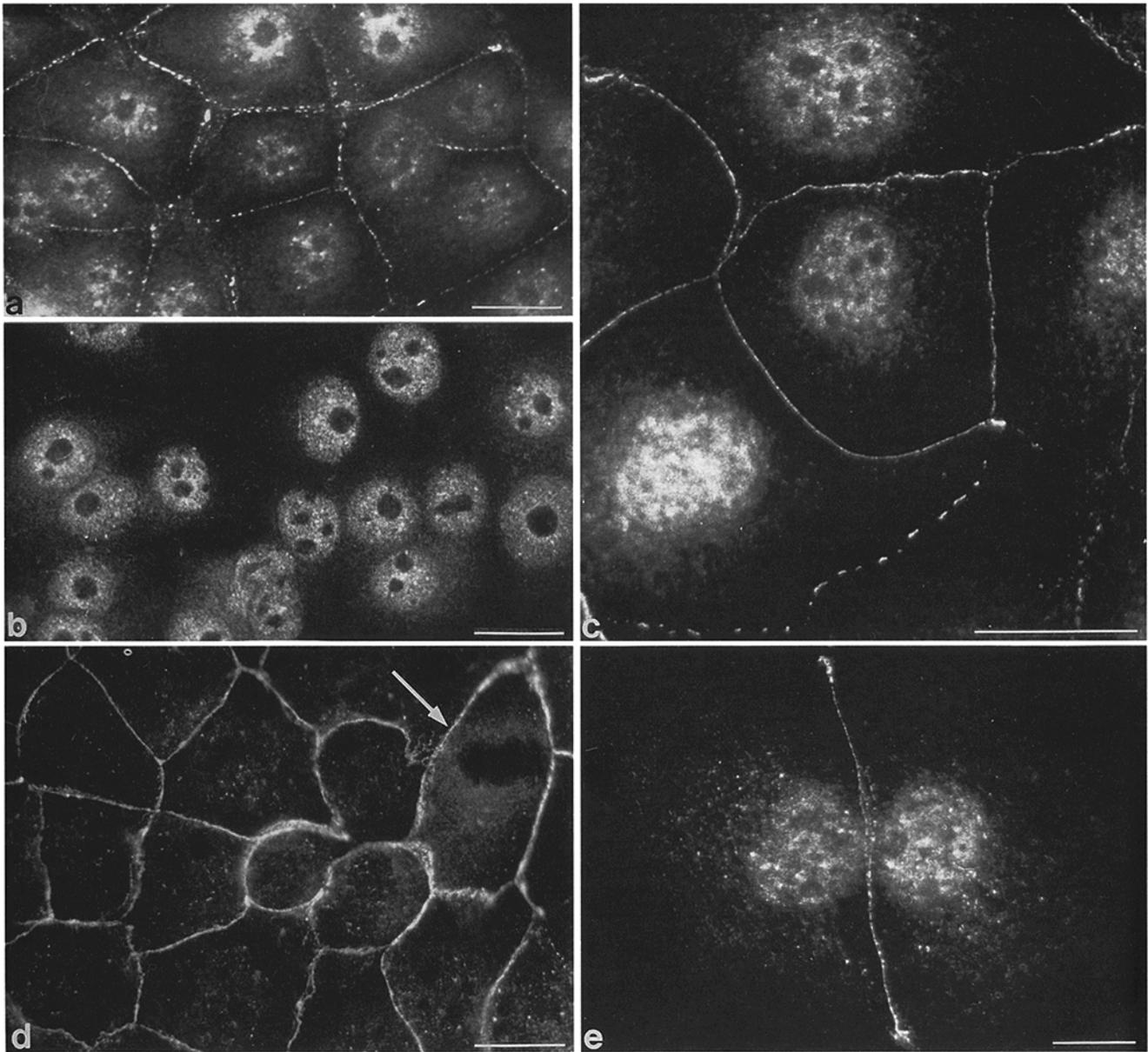
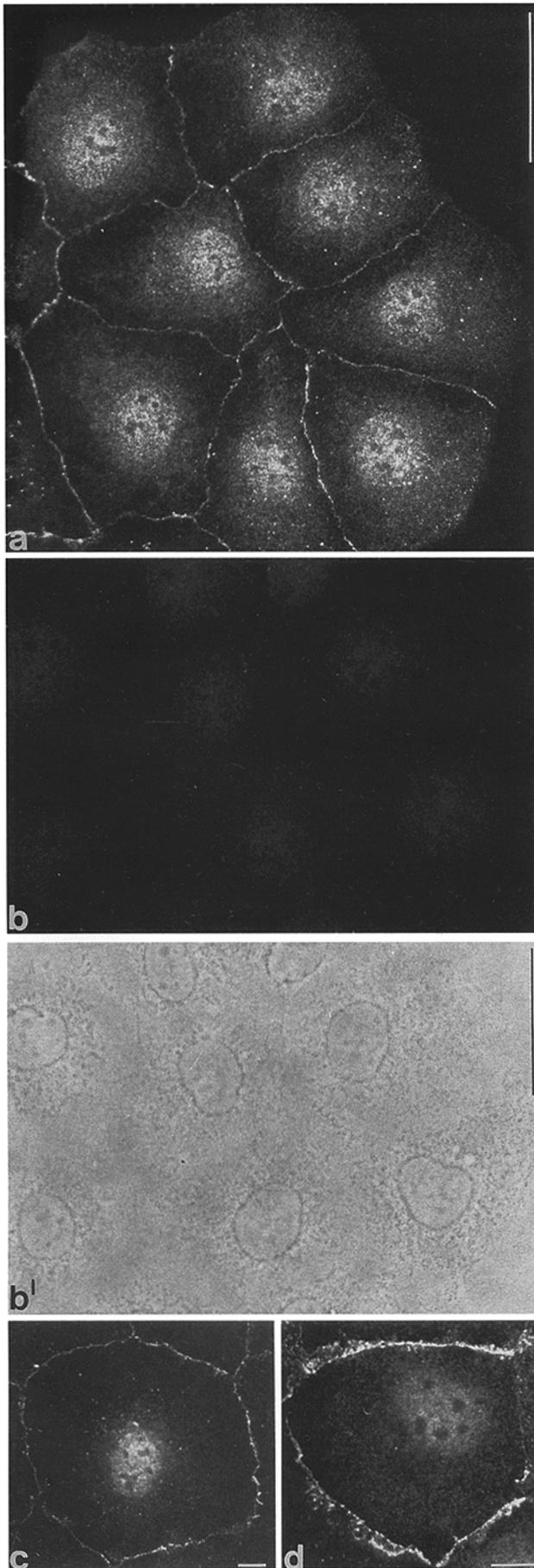


Figure 5. Immunofluorescence microscopy of plakophilin 2 in cultures of epithelial cells using polyclonal guinea pig antibodies. (a) Human MCF-7 cell monolayer treated in the presence of detergent according to protocol 1, showing specific staining of cell-cell boundaries in the typical punctate desmosomal pattern as well as patchy fluorescence in the karyoplasm. (b) Similar MCF-7 cell monolayer, however, reacted in the absence of detergent according to protocol 3. Under these conditions, exclusively intense nuclear staining is seen, probably due to masking of the plaque-bound form. Note absence of immunostaining from nucleoli. (c) Higher magnification of canine MDCK cell monolayer, treated according to protocol 1, showing the finely granular aspect of the karyoplasmic reaction. (d) Staining of cell borders in human PLC cell monolayer, after treatment according to protocol 1 but at prolonged time. Under these conditions, plakophilin 2 is largely "washed out" from the nucleus. Note that during mitosis (*arrow*) plakophilin 2 is transiently dispersed over the cytoplasm. (e) Postmitotic daughter cells (MDCK), treated according to protocol 1 as in c, showing besides granular immunostaining in the nucleus, the presence of plakophilin 2 in the very small nascent desmosomes at the cell-cell boundary, whereas free cell edges are negative. Bars, 20 μm .

2 mRNA that differed only by the insertion of a 132-nucleotide (44-aa) exon between the second and third repeat. The smaller cDNA variant, termed plakophilin 2a, was originally isolated from a library derived from cultured Caco-2 cells, whereas the larger form, plakophilin 2b, was originally identified in a human heart cDNA library. Both splice forms were then detected in cDNAs from several other cell type libraries as well.

Using the isolated cDNA clones in Northern blot analysis, we found a relatively broad band of mRNA of 5.3 kb in cultured human epithelial and carcinoma cells, in simple, complex and transitional epithelia, and even in nonepithelial cell lines and tissues (Fig. 3). The occurrence of both or only one of these splice forms in diverse cell types is currently under study at both the mRNA and the protein level.



Amino Acid Sequence Comparison of Plakophilins 2a and 2b with Plakophilin 1

The plakophilin 2 cDNA clones obtained encode a short splice form polypeptide of 837 aa and the longer splice variant of 881 aa, with calculated molecular masses of 92,750 and 97,410 D, respectively. As shown in Fig. 4, plakophilins 2a and 2b are homologous and show a very similar *arm*-repeat organization as plakophilin 1 (cf. references 25, 26, 66). Both polypeptides contain nine complete copies of the *arm*-motif, followed by a short carboxy terminal extension of only 11 (plakophilin 2) or 13 (plakophilin 1) aa.

Between the *arm*-repeats, plakophilins 1 and 2 show a high sequence similarity, i.e., a total of 42% identical aa and 60% similarity, including conservative aa exchanges. The individual repeats vary in their degree of similarity, with remarkably high conservation of 71 and 74% aa positions identical or conservatively exchanged in repeats 4 and 6. However, sequence similarity between the two polypeptides is not restricted to the *arm*-repeat domain but could also be detected in the amino terminal (“head”) domain, although this portion is much longer in plakophilin 2 (Fig. 4). These related motifs in the head domain, however, are somewhat isolated from each other, starting with a 21-aa stretch of DXS(S/T)LALPS(E/D)XK(L/M)KXXG(S/T)SGR. The 44-aa insertion in plakophilin 2b does not reveal any apparent sequence similarity to the *arm* motif.

Immunofluorescence Microscopy of Cultured Cells

To localize plakophilin 2, we performed immunostaining experiments on diverse cultured human cell lines using the polyclonal guinea pig as well as the monoclonal murine antibodies and different protocols. A typical linearly punctate desmosomal staining at cell–cell borders was found in all epithelial cell culture lines examined, including examples of cells from polarized (simple) epithelia and adenocarcinomas (e.g., Figs. 5, 6, *a–d*, and 8, *a* and *b*) as well as cells derived from stratified epithelia and their carcinomas such as the vulvar squamous carcinoma–derived line A-431 and HaCaT keratinocytes (results not shown). Plakophilin 2 antibodies also showed cross-reactivity with desmosomes of bovine cells such as the MDBK kidney and BMGE mammary gland lines (results not shown).

The punctate reaction sites decorated by plakophilin 2 antibodies were clearly identified as desmosomes by colocalization with desmoplakin antibodies (Fig. 8, *a* and *a'*), whereas adherens junction markers such as α - and β -catenin displayed a different staining pattern. This differential localization with respect to markers of other junction types such as adherens junctions (α - and β -catenin, E-cadherin, vinculin) and tight junctions (protein ZO-1, symplekin) was also demonstrated by laser scanning confocal microscopy (e.g., Fig. 8 *b*; see also reference 34).

Figure 6. Human liver carcinoma PLC cells treated according to protocol 1 but with murine mAbs PP2-77 (*a*), PP2-63 (*c*), or PP2-66 (*d*), showing both nuclear and desmosomal reactions, in comparison with a control in which only secondary antibodies have been applied (*b*, epifluorescence; *b'*, same field in phase contrast optics). Bars: (*a–b'*) 50 μ m; (*c* and *d*) 10 μ m.

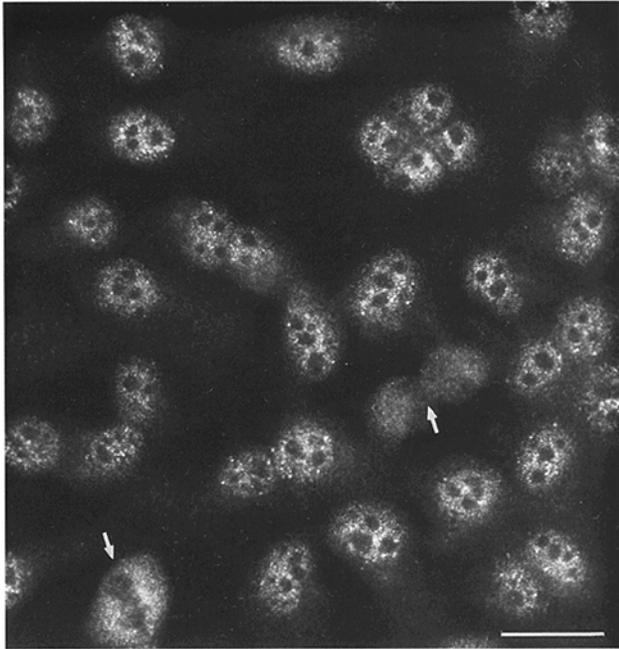
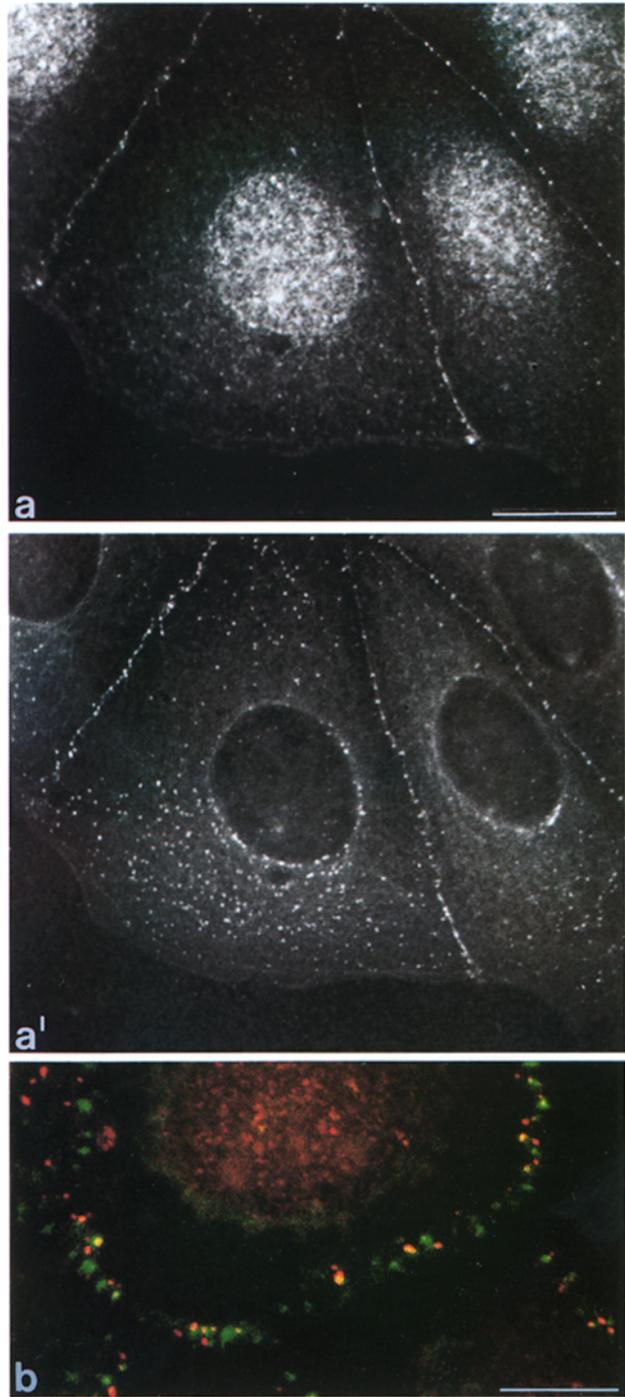


Figure 7. Immunofluorescence microscopy of SV-40-transformed human fibroblasts (“SV-80 fibroblasts”), processed according to protocol 3 (see Materials and Methods), presenting nuclear immunostaining, except in the mitotic cells (*arrows*) shown in which the protein is transiently spread throughout the cytoplasm. Bar, 25 μm .

Much to our surprise, we noticed intense immunoreaction of plakophilin 2 in the karyoplasm in all the cell cultures tested, leaving the nucleoli and condensed chromatin structures negative (Fig. 5, *a* and *b*). This nuclear immunoreaction was consistently observed with all our polyclonal antibodies and mAbs against plakophilin 2 (for example see Fig. 6, *a*, *c*, and *d*) but not in controls (e.g., Fig. 6, *b* and *b'*) and was clearly dependent on the specific preparation conditions: While after short incubation times and particularly without the use of detergent, a staining reaction was almost exclusively seen in the interphase nuclei (Fig. 5 *b*), more extended incubations, in combination with detergent treatment, revealed simultaneously the nuclear and desmosomal immunoreaction (Figs. 5, *a*, *c*, and *e* and 6 *a*). Sometimes prolonged incubation and washes in buffers containing detergents could lead to progressive loss of the karyoplasmic plakophilin 2 staining (Fig. 5 *d*). As such we empirically determined “intermediate” preparative conditions that allowed the simultaneous visualization of both the desmosomal and the nuclear reaction (e.g., Fig. 5, *a*, *c*, and *e*). Nonepithelial cell lines devoid of desmosomes, in-

Figure 8. Double-label immunofluorescence microscopy of plakophilin 2 (*a*), in comparison to desmoplakin (*a'*) and α -catenin (*b*), in cultured human epithelial cells treated according to protocol 1 (see Materials and Methods). (*a*) Double-label immunostaining of plakophilin 2 (*a*) with desmoplakin (*a'*) on freshly



plated PLC cells. Note detection of plakophilin 2 in the nucleus, in contrast to the nuclear absence of desmoplakin, while both proteins colocalize in the small, nascent desmosomes at cell-cell borders. (*b*) Laser scan confocal microscopy of double-label immunofluorescence showing a comparison of plakophilin 2 (*red*) with α -catenin (*green*) in cultured human A-431 cells. For the most part, plakophilin 2 and α -catenin do not colocalize, showing the absence of plakophilin 2 in the *puncta adherentia* containing catenins and E-cadherin. (It is not clear whether the few sites of overlap, as seen by the yellow color, are due to localization of both proteins in the same structure or the closely spaced occurrence of two different structures in the same volume element analyzed.) Again, note detection of plakophilin 2 in the nucleus. Bars: (*a*) 20 μm ; (*b*) 5 μm .

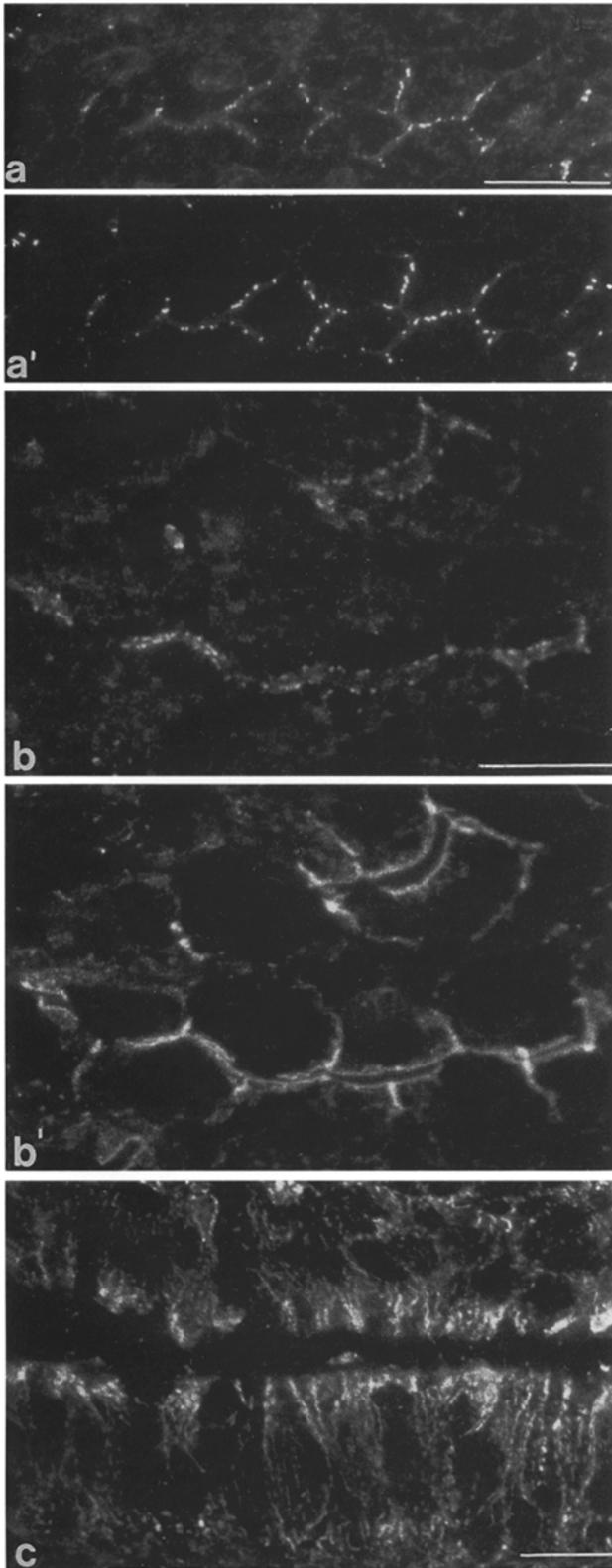


Figure 9. Immunolocalization of plakophilin 2 on cryostat sections through bovine liver (*a–b'*) and a human colon carcinoma (*c*) using protocol 1 (see Materials and Methods). (*a* and *a'*) Double-label immunofluorescence microscopy, showing typical desmosomal punctate array of plakophilin 2 along the bile canaliculi (*a*), which is indistinguishable from the immunostaining observed with desmoplakin antibodies (*a'*). (*b* and *b'*) Double labeling show-

cluding human SV-80 fibroblasts, showed only the karyoplasmic reaction (Fig. 7). The nuclear localization was also demonstrated by double-label experiments using certain nuclear proteins and DNA as markers, including 4', 6-diamino-2-phenylindole (DAPI) dye (results not shown).

In all these cell cultures, mitotic cells were characterized by a spreading of the immunofluorescent plakophilin 2 over the cytoplasm (Figs. 5 *d* and 7, *arrows*). Telophase and early G1-phase cells already had accumulated the protein in the karyoplasms of the daughter nuclei. It was also clear that the nuclear plakophilin 2 localization was not significantly different throughout the cell cycle and in response to cell density but was a ubiquitous and constitutive feature seen with both the mono- and polyclonal antibodies used. The specificity of the intense nuclear immunoreaction of plakophilin 2 was further demonstrated in double-label experiments, in comparison with, for example, desmoplakin (Fig. 8 *a'*) as well as α - and β -catenins (Fig. 8 *b*).

Immunofluorescence Microscopy of Tissues

The desmosomal location of plakophilin 2 was also evident from our extensive immunofluorescence microscopical studies, using sections through epithelial tissues and standard protocols. All simple and complex epithelia showed plakophilin 2 in punctate arrays which in double-label experiments were also positive for desmoplakin but not for adherens junction markers such as α - and β -catenin or E-cadherin, as is presented for bovine liver in Fig. 9, *a–b'*. This was also true for human liver, stomach, intestine, pancreas, and skin glands and all tumors derived from simple epithelia (Fig. 9 *c* shows a differentiated colon carcinoma with pronounced subapical concentration of desmosomes) and bladder carcinomas.

Intense plakophilin 2 immunostaining of desmosomes was also observed in certain stratified squamous epithelia. While this reaction was practically restricted to the basal cell layer of some tissues, such as vagina, and seemed to be absent in suprabasal cell layers of esophagus, tongue, and epidermis (not shown; see also below), we observed intense immunostaining at cell borders in all living cell layers of the perinasal epithelium of bovine muzzle (Fig. 10). Double-label immunofluorescence microscopy further revealed that here most of the plakophilin 2 staining appeared to be concentrated at the internal face of the plaque, relative to desmoplakin reaction that was closer to the membrane (Fig. 10).

Again, with the appropriate incubation times and particularly after aldehyde fixation, plakophilin 2 could also be localized to nuclei of tissue sections. This was observed in various epithelia and nonepithelial tissues. For example, reactions on bovine tongue mucosa and muzzle epidermis

ing that the arrays stained with plakophilin 2 antibodies (*b*) differs markedly from the reaction of the adherens junction plaque marker, β -catenin (*b'*). (*c*) In human colon carcinoma, plakophilin 2-positive reaction sites are concentrated in the subapical ring of desmosomes but also occur in the sparser desmosomes along the lateral walls. Note also that under these conditions nuclear plakophilin 2 seems to have been lost. Bars, 20 μ m.

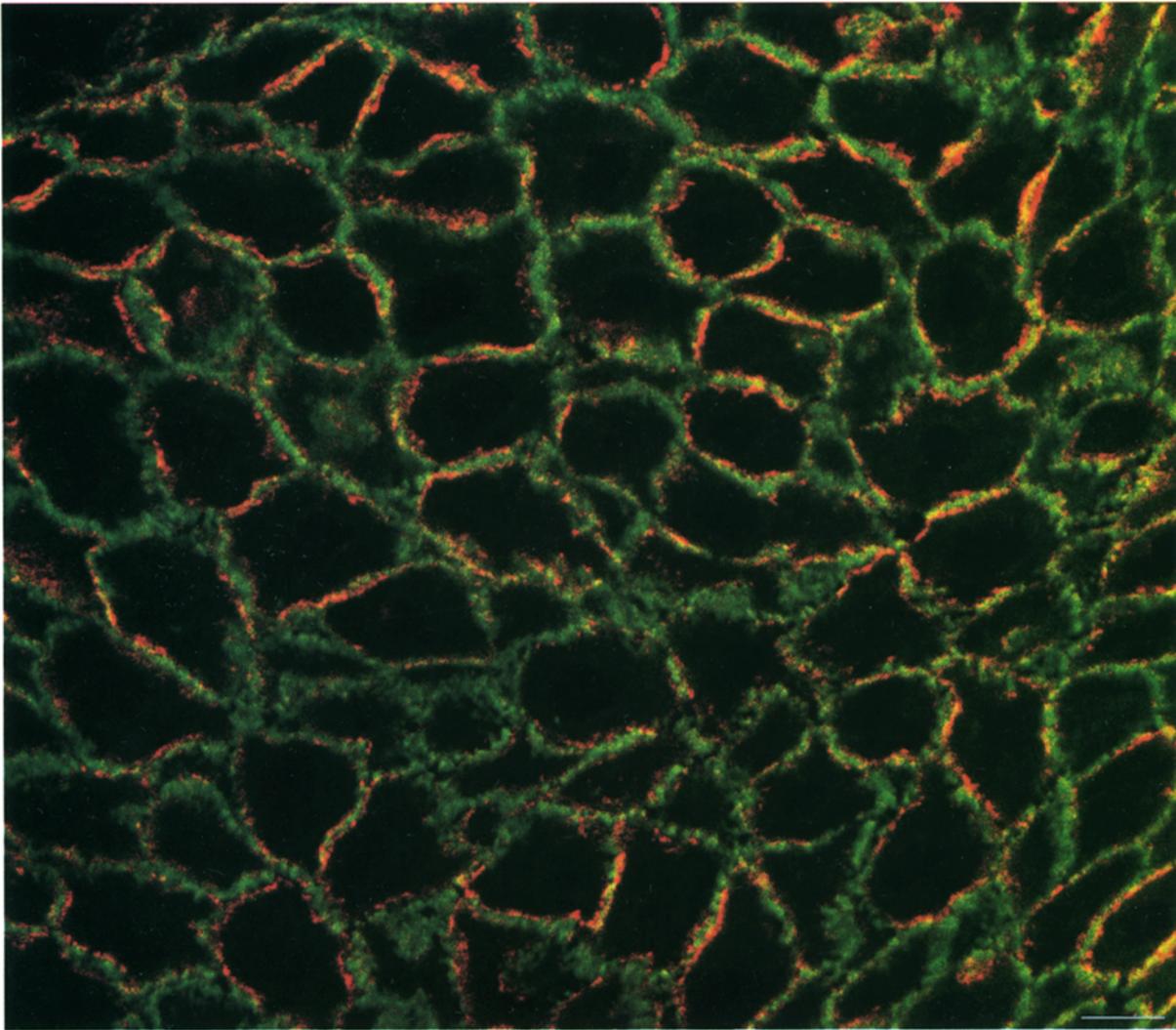


Figure 10. Laser scanning confocal microscopy showing double-label immunofluorescence localization of plakophilin 2 (*red*) and desmoplakin (*green*) on bovine muzzle epithelium. The two desmosomal plaque components are closely associated but do not colocalize. The plakophilin 2 reaction sites are mostly located inside of the desmoplakin-positive structures. Bar, 10 μm .

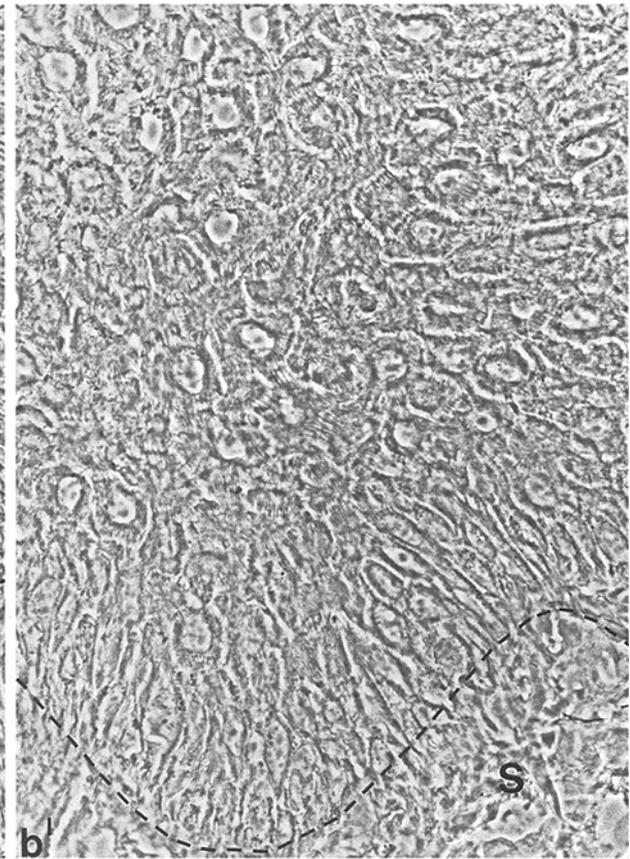
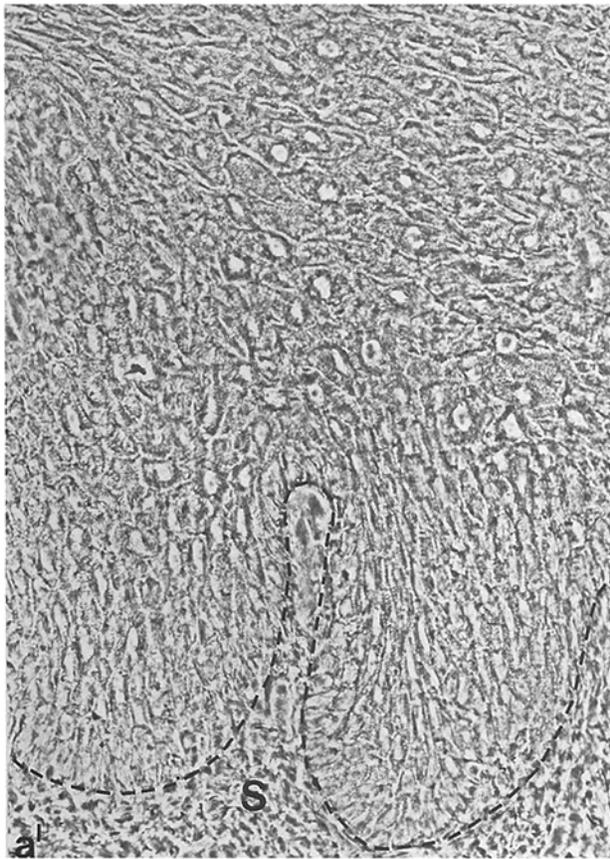
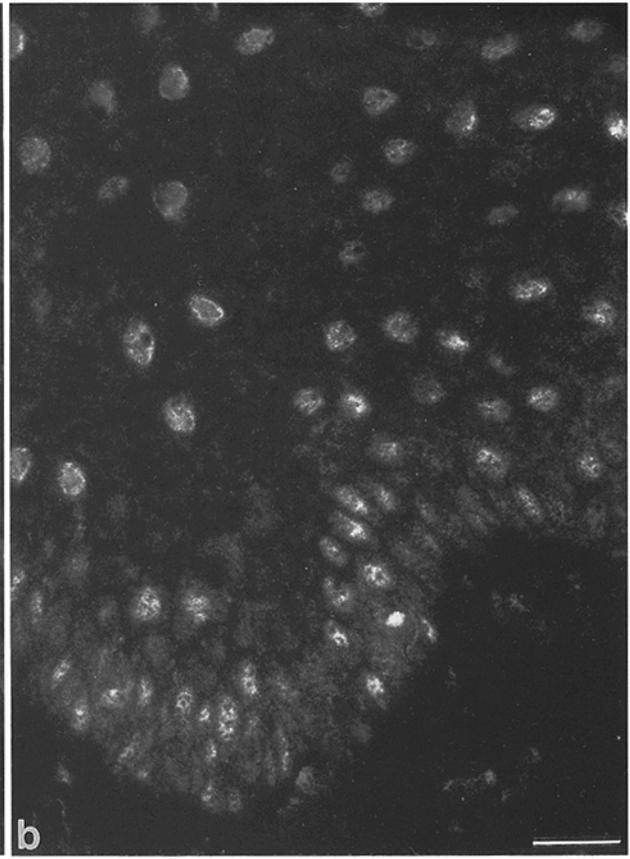
are shown in Figs. 11 and 12. In the diverse stratified tissues examined, plakophilin 2-positive immunostaining was more frequently seen in nuclei of basal cell layers, whereas a considerable proportion of nuclei appeared negative in upper strata (Fig. 11). Intense and specific plakophilin 2 immunoreaction was also seen in the nuclei of glandular ducts and acini (Fig. 12, *a-c'*), thus demonstrating nuclear plakophilin 2 in diverse types of epithelial cells, including simple epithelial ones (Fig. 12, *c* and *c'*). Moreover, the nuclear plakophilin 2 reaction could also be

detected in nonepithelial stromal cells such as fibroblasts and endothelial cells of submucosa (Fig. 12 *b*).

Immunofluorescence Microscopy of Nonepithelial Desmosomes

Our finding in Northern blots and immunoblots that the plakophilin 2 gene is also expressed in certain nonepithelial tissues such as heart and lymph nodes was confirmed and visualized by immunofluorescence microscopy of bo-

Figure 11. Immunofluorescence microscopy showing the nuclear localization of plakophilin 2 on cryostat sections through bovine stratified epithelia after formaldehyde fixation (see Materials and Methods). (*a*) Tongue mucosa reacted according to protocol 1. (*a'*) Phase contrast optics of the same field. (*b*) Muzzle epithelium treated according to protocol 2. (*b'*) Phase contrast. Intense and specific nuclear immunostaining is seen that appears to be more frequent in the basal cell layers (*a*, survey picture; *b*, higher magnification). The *dashed lines* in *a'* and *b'* denote the level of the basal lamina; *S*, submucosa. Bars: (*a*) 50 μm ; (*b*) 25 μm .



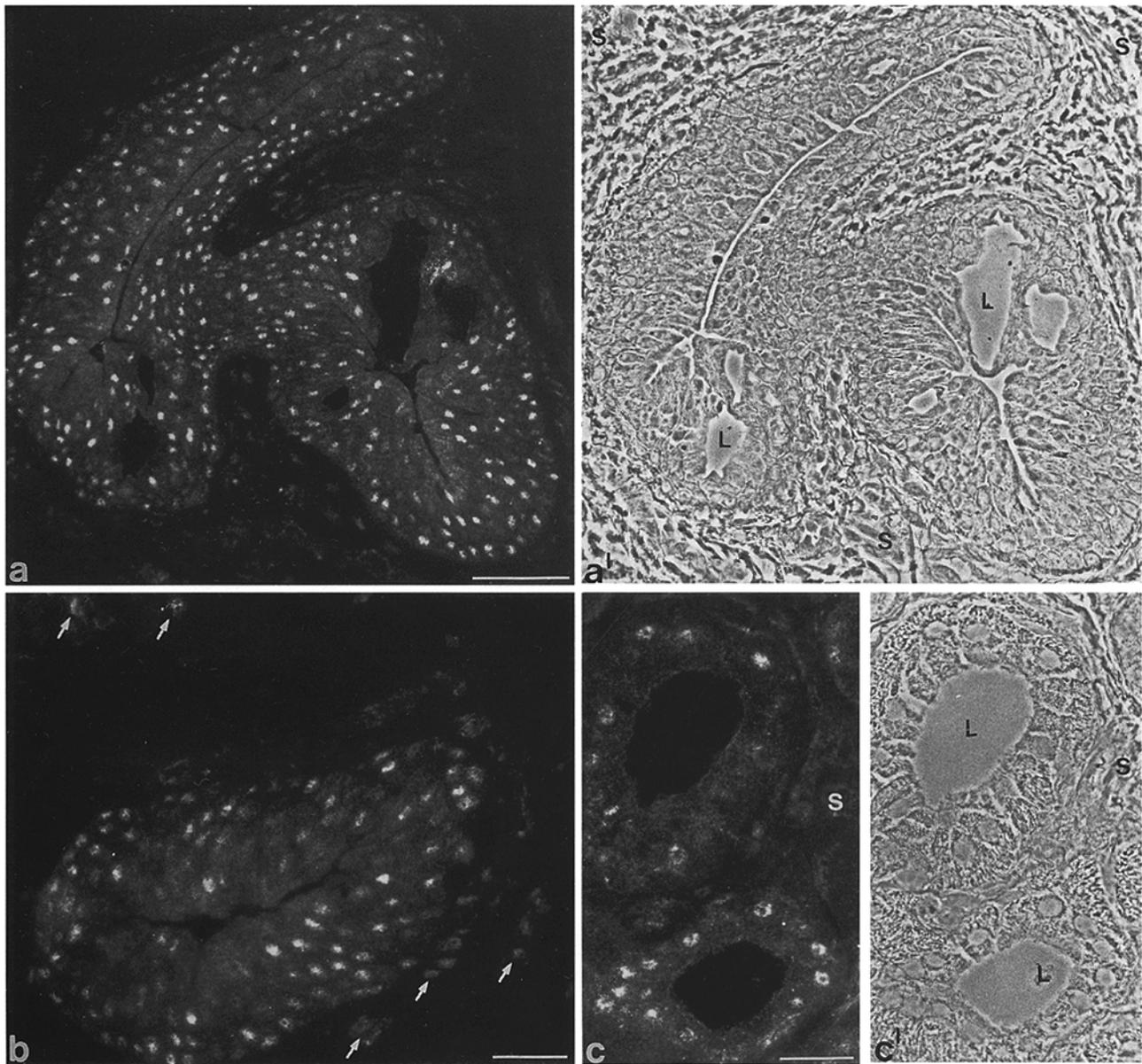


Figure 12. Nuclear plakophilin 2 immunostaining in glandular ducts and acini shown by reaction on tissue sections through bovine muzzle epithelium fixed with formaldehyde (see Materials and Methods). (a) Survey picture, showing intense nuclear reaction of the epithelial cells of a complex glandular duct. (a') Phase contrast. L, lumen; S, submucosa. (b) Higher magnification of a glandular duct demonstrating that nuclear accumulation of plakophilin 2 is not restricted to epithelial cells but can also be detected in the mesenchymal cells of the surrounding stroma (arrows). (c) Nuclear immunostaining in the simple acinar epithelia of acini. (c') Phase contrast of the same optical field. L, lumen; S, submucosa. Bars: (a) 50 μm ; (b and c) 25 μm .

vine and human tissues. As shown in Fig. 13, *a* and *a'*, the desmosomes in the intercalated disks connecting cardiomyocytes were intensely and uniformly decorated by plakophilin 2-specific antibodies (some intercalated disks are denoted by arrows in Fig. 13 *a'*.) Moreover, the desmosomes connecting the Purkinje fiber cells were also positive for plakophilin 2, displaying the same linear-punctate immunostaining as, for example, with desmoplakin antibodies (Fig. 13, *b* and *b'*). By contrast, the endothelial junctions of cardiac blood vessels, identified by their positivity for plakoglobin (Fig. 13 *c'*, cf. reference 17), were totally negative for plakophilin 2 (Fig. 13 *c*), and this was also true for diverse other types of blood vessels.

The positive plakophilin 2 reaction in lymph nodes was identified to be specific for the desmosomes connecting the cell processes of the dendritic reticulum of germinal centers, and these finely dotted arrays were also seen in the dendritic reticulum cells of lymphatic germinal centers in other tissues such as in the follicles of the mucosae of the gastrointestinal tract (not shown).

Immunoelectron Microscopy

In several epithelial tissues and cultured cells, we could verify the location of plakophilin 2 in the plaques of desmosomes by immunoelectron microscopy, using immu-

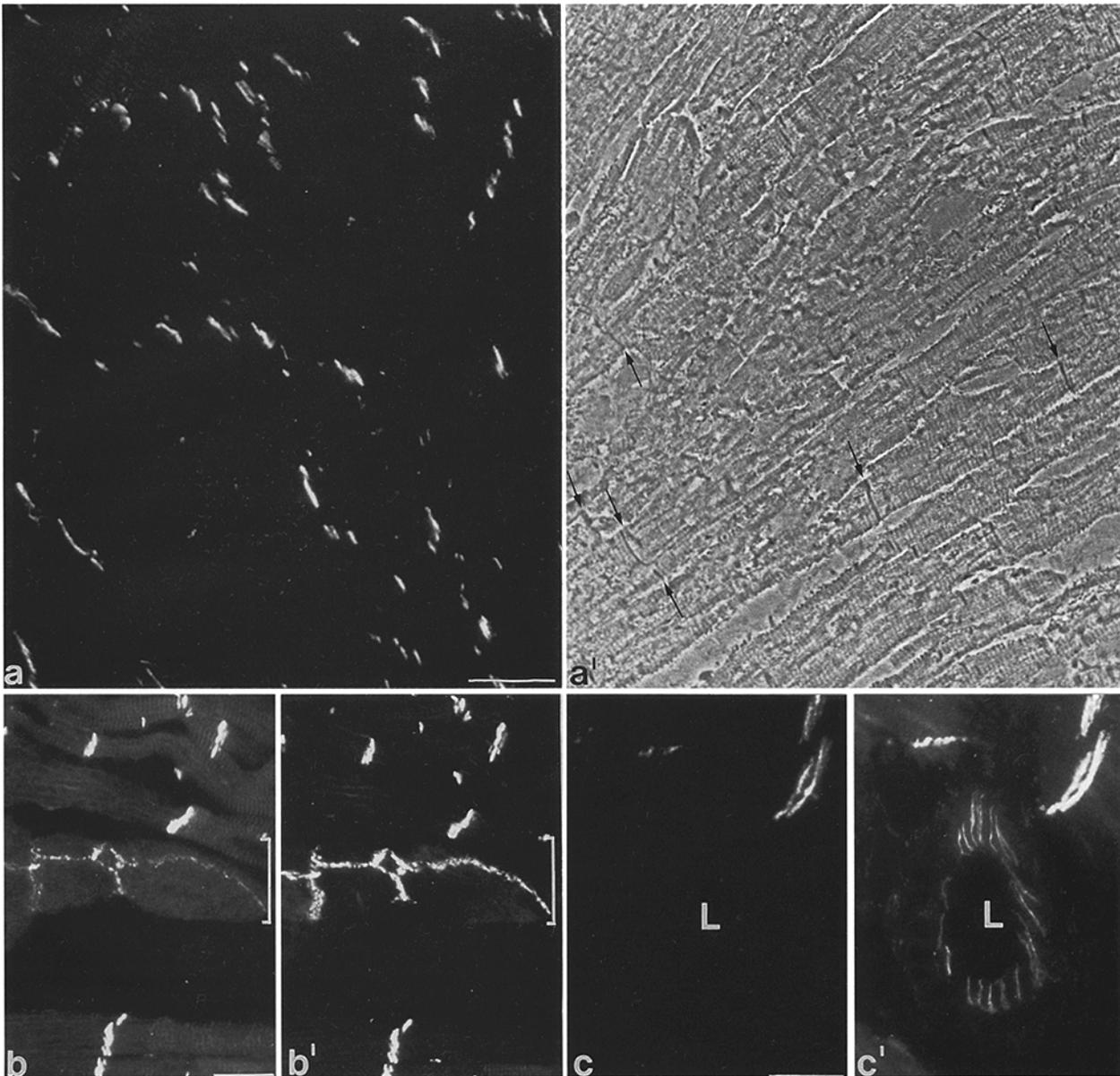
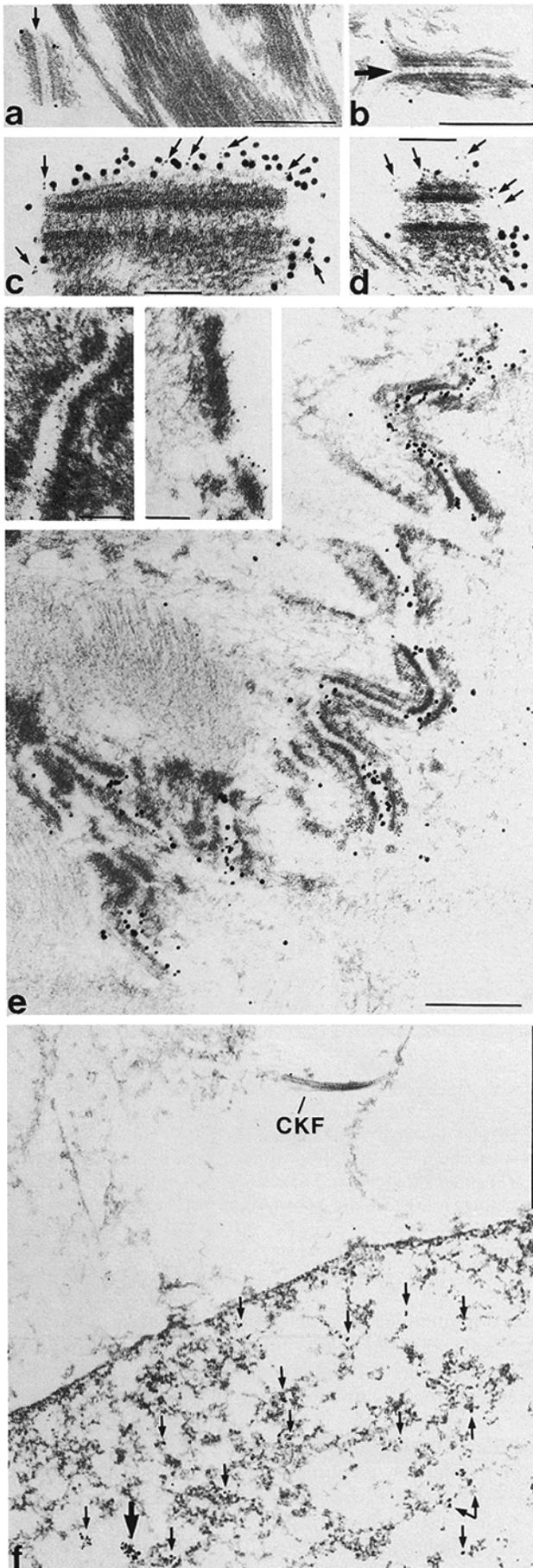


Figure 13. Immunofluorescence microscopy of cryostat sections through bovine heart, identifying plakophilin 2 as a prominent desmosomal component in the intercalated disks of the myocardium and the desmosomes of Purkinje fiber cells. (*a* and *a'*) Survey picture showing the localization of plakophilin 2 to the intercalated disks of myocardium. (*a'*) Phase contrast, picture of the same optical field (arrows, some intercalated disks). (*b* and *b'*) In double-label immunostaining, plakophilin 2 (*b*) and desmoplakin (*b'*) colocalize at cell-cell contacts in the Purkinje fiber bundles (middle, bracket) as well as in the adjacent intercalated disks (top and bottom). (*c* and *c'*) Double-label immunolocalization comparing the colocalization of plakophilin 2 (*c*) and plakoglobin (*c'*) in desmosomes of intercalated disks (upper right), in contrast to the absence of plakophilin 2 in endothelial junctions (*c*), which are known to be rich in plakoglobin. (*c'*) Central part. *L*, lumen of arteriole. Bars, 25 μm .

nogold label with or without silver enhancement and also in colocalization experiments with desmoplakin (e.g., Fig. 14, *a-d*). The specificity of the desmosomal location was also well demonstrable in the intercalated disks of the heart where all desmosomes were intensely labeled while the numerous interspersed *fasciae adhaerentes* and gap junctions were negative (Fig. 14 *e*).

However, while in the epithelial desmosomes most of the plakophilin 2 label was found near the cytoplasmic side of the plaque (Fig. 14, *a-d*), the cardiac desmosomes

showed immunolabel particle enrichment at or in the desmoglea, i.e., the seemingly “intercellular space.” Quantitation by counting the immunogold particles in different distances from the plaque revealed that in cardiac tissue, 88% of all immunogold particles were within a 20-nm distance from the membrane level in or at the desmoglea. This type of immunolabel was observed upon silver enhancement (Fig. 14 *e*) as well as with 5-nm gold particles (Fig. 14 *e*, left inset) and also at the artificial “surface” of half-desmosomes (compare also reference 10) formed by desmosome



splitting during freezing and thawing (Fig. 14 *e*, right inset). The desmoglea-oriented immunolocalization in cardiac desmosomes could be taken as indication that in this tissue the specific epitope of plakophilin 2 is located—or only accessible—in a mode different from that of epithelial desmosomes, and perhaps is very near the membrane. Clearly, the special topology of plakophilin 2 domains in the desmosomal plaques of the various tissues needs to be specifically examined in greater detail.

The nuclear enrichment of plakophilin 2 was also confirmed by immunoelectron microscopy (Fig. 14 *f*), showing immunogold particles scattered over the karyoplasm, except the nucleolus, with occasional clusters at certain small nuclear bodies that have not yet been identified (Fig. 14 *f*).

Discussion

The present study has revealed a new, widespread desmosomal protein, plakophilin 2, which appears in two variants, 2a and 2b, and also a new topogenic principle, i.e., the occurrence of a protein in two specific but distinct and distant cell structures, the karyoplasm and the desmosomal plaque.

Clearly, plakophilin 2 has to be added to the group of desmosome-specific plaque proteins (see introduction). It appears to be the only plakophilin detectable in the desmosomes of a wide range of cell types, including all simple and glandular epithelia but also nonepithelial tissues such as the myocardium and the Purkinje fibers of the heart and the dendritic reticulum of lymphatic germinal centers.

Figure 14. Electron microscopic localization of plakophilin 2 to the desmosomes of bovine muzzle epithelium (*a–d*) and of myocardium (*e*), and in the karyoplasm (*f*). (*a–d*) Desmosomes in cryosections through bovine muzzle epithelium showing plakophilin 2 antibodies (from guinea pig) reacted with 1-nm immunogold particles, followed by silver enhancement, at desmosomal plaques (arrows in *a* and *b*), and without silver enhancement, in double label examination together with desmoplakin mAbs, reacted with 10-nm immunogold particles (*c* and *d*, in which the small size plakophilin 2 label is denoted by small arrows). (*e*) Cryosection through bovine heart, showing the localization of plakophilin 2 to the mesogleal side of desmosomal plaques of the intercalated disks of the myocardium, whereas *fasciae adherentes*, anchoring the actomyosin fiber bundles, are practically negative (secondary reaction with 1-nm gold-coupled antibodies, followed by silver enhancement). The preferential labeling at the desmogleal membrane, which is also demonstrated by using 5-nm gold-coupled antibodies without enhancement (*left inset*), and on the artificial surface of half-desmosomes resulting from artificial desmosome splits (*right inset*), suggests that here the epitopes reactive are located near the membrane and the molecule is arranged differently from its organization in the epithelial desmosomes (*a–d*). (*f*) In the nucleus of cultured human PLC cells, plakophilin 2 is enriched and dispersed throughout the karyoplasm (arrows), with occasional clusters of plakophilin 2 staining at certain small karyoplasmic “bodies” (e.g., at the two arrows in the lower left), whereas little label is detected in the cytoplasm (CKF, cytokeratin filament bundle). Note that here the specimens had to be treated with the detergent saponin, resulting in some loss of cytoplasmic ground substance. Bars: (*a* and *b*) 0.5 μm , (*c* and *d*) 0.1 μm , (*f*) 1 μm , (*insets* in *e*) 0.2 μm .

As it can also be detected in desmosomes of embryonic and embryonal carcinoma cells (results not shown), and as plakophilin 1 has only been found in the desmosomes of certain stratified and complex epithelia (26, 31, 66), we view plakophilin 2 as the more general and fundamental constituent of the desmosomal plaque. Moreover, we conclude that plakophilins as a protein type are constitutive desmosomal elements: A given desmosome contains either plakophilin 1 or 2 or both. Consequently, desmosomes can differ, in cell type-characteristic patterns, not only by their complements of desmogleins (Dsg1-3) and desmocollins (Dsc1-3; cf. references 35, 38, 39, 44, 53, 64) but also by their specific plakophilin complement.

In this context, we have also to emphasize our finding that both plakophilins 1 and 2 can coexist in the desmosomes of certain tissues such as in cow snout epithelium, the "classic" tissue of choice for isolating desmosomes. Why then has plakophilin 2 escaped the detection in our and other author's previous studies on bovine muzzle desmosomes or desmosome-enriched fractions from other cells (see, e.g., references 5, 6, 11, 14-16, 22, 50, 70, 71; for reviews see references 7, 21, 68, 69)? The most plausible explanation is that plakophilin 2 differs from the other desmosomal components by its relatively higher extractability, as also shown in the present paper, and therefore might have been selectively lost during the numerous extractions and washes, partly in rather harsh media used to isolate pure desmosomal structures.

Among the *arm*-repeat-containing proteins, including several junctional plaque components, such as the *armadillo* gene product, plakoglobin, β -catenin, and protein p120, the plakophilins 1 and 2a and 2b are closely related to each other, representing a plakophilin subgroup. Some *arm*-repeat-containing proteins have been experimentally shown to form specific and stable complexes with certain cadherins, e.g., plakoglobin with desmosomal cadherins (4, 45, 75-77; for β -catenin see also references 9, 28, 29, 37, 52, 55, 61, 62), and this cadherin binding has been localized to specific segments of the *arm*-repeat domain. Obviously, plakophilins are now also candidates to be examined for direct cadherin binding. Furthermore, as plakophilin 1 is known to bind cytokeratins specifically and avidly (25, 31), plakophilins 2a and 2b may also be cytokeratin-binding molecules. This hypothesis is currently under examination in our laboratory, as we are also attempting to elucidate, using assays involving recombinant DNA mutagenesis, the functional significance of the much longer head domain of plakophilins 2a and 2b and the possible importance of the 44-aa insertion characteristic of plakophilin 2b.

It is also clear from the present study that plakophilin 2 is not synthesized in relation to epithelial differentiation or to cytokeratin filament formation; its presence is not even correlated with desmosome formation. Quite to the contrary, plakophilin 2 is found in sizable amounts in diverse nonepithelial, non-desmosome-forming cells growing in culture and also in various tissues lacking desmosomes. This suggests that plakophilin 2 serves at least one function outside of the desmosomal context.

Here, our general finding that plakophilin 2 is often located, in desmosome-possessing as well as desmosome-lacking cells, in the nucleus where it is apparently spread over the interchromatinic karyoplasm is intriguing. Conse-

quently, the interphase nucleus is the only cell structure in which this protein is significantly enriched in cells devoid of desmosomes. In response to recent discussions that cell population density or the proliferation state may determine the nucleocytoplasmic distribution of certain proteins such as the tight junction-associated plaque protein ZO-1 (23) and the product of the gene suppressing the von Hippel-Lindau multiple tumor syndrome (43), we have here to emphasize that plakophilin accumulation in nuclei can be observed in growth-arrested as well as in proliferating cells, in sparse as well as in highly confluent cultures. Unlike another *arm*-repeat protein, pendulin, the product of the tumor suppressor *oho3* gene in *Drosophila* (42, 74), which is translocated into the karyoplasm only in late G2-interphase, the nuclear accumulation of plakophilin 2 is obviously not cell cycle dependent. We therefore conclude that plakophilin 2 is a constitutive nuclear protein with an as yet unidentified nuclear function. The existence of a mechanism rapidly and efficiently "pumping" plakophilin 2 into the nucleus is particularly evident from its reaccumulation in mitotic telophase from a transiently dispersed cytoplasmic state. Presently, we cannot say whether this nuclear accumulation involves a "nuclear localization signal" (NLS) or whether plakophilin 2 is transported into the nucleus in a "piggy-back" complex with a NLS-containing protein (for a review on possible mechanisms see reference 48).

The massive nuclear accumulation of plakophilin 2 has been missed in the initial phase of our study as it is rather soluble and thus readily lost from cryostat sections or lysed cells of monolayer cultures during extractive procedures such as prolonged incubations in aqueous media and detergent treatments. Only when precautions were taken in our immunocytochemical protocols to optimize the retention of soluble nuclear proteins (for general problems of artificial extraction and redistribution see, e.g., references 47, 56), as we in our laboratory had experienced them from previous work with soluble karyoplasmic proteins (cf. references 3, 40, 41), we consistently visualized the nuclear pool of plakophilin 2.

While plakophilin 2 is the first adhering junction plaque protein found to accumulate naturally in the nucleus, it has to be remembered that the appearance of *arm*-repeat plaque proteins in nuclei of *Xenopus* embryos has been reported for experimental situations of overproduction of slightly modified β -catenin and plakoglobin, induced by injection of mRNAs synthesized in vitro (20, 33). These observations and related findings of a role of the *armadillo* gene product of *Drosophila* in the *wingless* signaling pathway have led to the widely shared hypothesis that β -catenin as well as plakoglobin not only serve a structural function in the specific junction but also may be more directly involved in the gene regulatory transduction of cell-cell interactions by migrating into the nucleus and/or retour (for reviews see references 24, 57, 73). Our present study now adds plakophilin 2, a protein naturally and constitutively occurring in nuclei and in desmosomal junctions, as an especially strong candidate to the list of *arm*-repeat-containing proteins with a possible function in plasma membrane \leftrightarrow cell nucleus signal transduction pathways.

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