

# Cross-Talk between Adherens Junctions and Desmosomes Depends on Plakoglobin

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**Abstract.** Squamous epithelial cells have both adherens junctions and desmosomes. The ability of these cells to organize the desmosomal proteins into a functional structure depends upon their ability first to organize an adherens junction. Since the adherens junction and the desmosome are separate structures with different molecular make up, it is not immediately obvious why formation of an adherens junction is a prerequisite for the formation of a desmosome. The adherens junction is composed of a transmembrane classical cadherin (E-cadherin and/or P-cadherin in squamous epithelial cells) linked to either  $\beta$ -catenin or plakoglobin, which is linked to  $\alpha$ -catenin, which is linked to the actin cytoskeleton. The desmosome is composed of transmembrane proteins of the broad cadherin family (desmogleins and desmocollins) that are linked to the intermediate filament cytoskeleton, presumably through plakoglobin and desmoplakin. To begin to study the role of adherens junctions in the assembly of

desmosomes, we produced an epithelial cell line that does not express classical cadherins and hence is unable to organize desmosomes, even though it retains the requisite desmosomal components. Transfection of E-cadherin and/or P-cadherin into this cell line did not restore the ability to organize desmosomes; however, overexpression of plakoglobin, along with E-cadherin, did permit desmosome organization. These data suggest that plakoglobin, which is the only known common component to both adherens junctions and desmosomes, must be linked to E-cadherin in the adherens junction before the cell can begin to assemble desmosomal components at regions of cell–cell contact. Although adherens junctions can form in the absence of plakoglobin, making use only of  $\beta$ -catenin, such junctions cannot support the formation of desmosomes. Thus, we speculate that plakoglobin plays a signaling role in desmosome organization.

SQUAMOUS epithelial cells typically contain two prominent types of cell–cell junctions: the adherens junction and the desmosome. The adherens junction is an intercellular adhesion complex that is composed of a transmembrane protein (a classical cadherin) and numerous cytoplasmic proteins ( $\alpha$ -catenin,  $\beta$ -catenin and plakoglobin, vinculin and  $\alpha$ -actinin; for reviews see Takeichi, 1990; Geiger and Ayalon, 1992). The cadherins are directly responsible for adhesive interactions via a  $\text{Ca}^{2+}$ -dependent, homotypic mechanism, i.e., in the presence of sufficient  $\text{Ca}^{2+}$ , cadherin on one cell binds to an identical molecule on an adjacent cell. The desmosome, also an intercellular

adhesion complex, is composed of at least two different transmembrane proteins (desmoglein and desmocollin) as well as several cytoplasmic proteins, including desmoplakins and plakoglobin (Koch and Franke, 1994). The transmembrane components of the desmosome are members of the broadly defined cadherin family and also require  $\text{Ca}^{2+}$  for adhesive activity. However, decisive experimental evidence for homophilic or heterophilic interactions between desmosomal cadherins via their extracellular domains has not yet been presented (Koch and Franke, 1994; Kowalczyk et al., 1996). While members of the cadherin family constitute the transmembrane portion of both adherens junctions and desmosomes, the different classes of cadherins are linked to different cytoskeletal elements by the cytoplasmic components of each junction. Specifically, the classical cadherins are linked to actin filaments and the desmosomal cadherins to intermediate filaments.

The organization of the proteins within the adherens junction is well understood (for reviews see Kemler, 1993; Cowin, 1994; Wheelock et al., 1996). Specifically, the intra-

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cellular domain of cadherin interacts directly with either plakoglobin or  $\beta$ -catenin, which in turn binds to  $\alpha$ -catenin (Jou et al., 1995; Sacco et al., 1995).  $\alpha$ -Catenin interacts with  $\alpha$ -actinin and actin filaments, thereby linking the cadherin/catenin complex to the cytoskeleton (Knudsen et al., 1995; Rimm et al., 1995). Cadherin/catenin complexes include either plakoglobin or  $\beta$ -catenin but not both (Näthke et al., 1994).

The importance of the classical cadherins to the formation of adherens junctions and desmosomes has been demonstrated. Keratinocytes maintained in medium with low  $\text{Ca}^{2+}$  (i.e., 30  $\mu\text{M}$ ) grow as a monolayer and do not exhibit adherens junctions or desmosomes; however, elevation of  $\text{Ca}^{2+}$  concentration induces the rapid formation of adherens junctions followed by the formation of desmosomes (Hennings et al., 1980; Tsao et al., 1982; Boyce and Ham, 1983; Hennings and Holbrook, 1983; O'Keefe et al., 1987; Wheelock and Jensen, 1992; Hoidalva and Watt, 1994; Lewis et al., 1994). Simultaneous blocking with function-perturbing antibodies against the two classical cadherins (E- and P-cadherin) found in keratinocytes inhibits not only  $\text{Ca}^{2+}$ -induced adherens junction formation but also severely limits desmosome formation (Lewis et al., 1994; Jensen et al., 1996). Consistent with these findings, expression of a dominant-negative cadherin by keratinocytes results in decreased E-cadherin expression and delayed assembly of desmosomes (Fujimori and Takeichi, 1993; Amagai, et al., 1995). These data suggest some form of cross-talk between the proteins of the adherens junction and those of the desmosome. One candidate protein that might mediate such cross-talk is plakoglobin, since it is the only known common component of both junctions.

Plakoglobin is found to be associated with the cytoplasmic domains of both the classical cadherins and the desmosomal cadherins. Despite the high degree of identity between plakoglobin and  $\beta$ -catenin (65% at the amino acid level; Fouquet et al., 1992),  $\beta$ -catenin only associates with the classical cadherins and not with the desmosomal cadherins. In the adherens junction, plakoglobin and  $\beta$ -catenin have at least one common function, i.e., the linking of cadherin to  $\alpha$ -catenin and thus to actin. However, there is emerging evidence that other functions of these two proteins are not identical. For example, in a study by Navarro et al. (1993), E-cadherin transfected into a spindle cell carcinoma was shown to associate with  $\alpha$ - and  $\beta$ -catenin, but not with the low levels of endogenous plakoglobin. The transfected cells did not revert to a more epithelial morphology in spite of the presence of functional E-cadherin, and the authors suggested that the lack of plakoglobin may have prevented such morphological reversion.

In the present study, we have tested the hypothesis that plakoglobin, through its interaction with E- or P-cadherin, serves as a regulatory molecule for desmosome organization. Even though plakoglobin is not an essential structural component of the adherens junction (Sacco et al., 1995), our data indicate that plakoglobin can function as a regulator of desmosome formation only when it is associated with a classical cadherin. Thus, we propose that plakoglobin has at least two functions: (a) as a structural component of the adherens junction and the desmosome and (b) as a signaling molecule that regulates communication between the adherens junction and the desmosome.

## Materials and Methods

### Reagents

Unless otherwise stated, all reagents were from Sigma Chemical Co. (St. Louis, MO).

### Cell Culture

The human epidermoid carcinoma cell line A431 was obtained from American Type Culture Collection (Rockville, MD) and maintained in DME (Gibco Laboratories, Grand Island, NY), 5% FCS (Hyclone Laboratories, Logan, UT), glutamine at 540 mg/L (Gibco Laboratories), and antibiotics (Penicillin-Streptomycin) at 50 U penicillin and 50 mg streptomycin/L (Gibco Laboratories). Rat 2 cells and Wnt 1-expressing Rat 2 cells were kindly provided by Dr. Anthony M.C. Brown (Cornell University Medical College, New York) (Jue et al., 1992) and maintained in DME, 5% FCS.

### Derivation of A431D Cells

A431 cells were placed into DME, 5% FCS containing  $10^{-7}$  M dexamethasone and grown until confluent. Cells were then lightly trypsinized to recover approximately half the cell population; trypsin was neutralized with FCS, and the cells were centrifuged and resuspended in fresh medium, still containing dexamethasone. This procedure was continued for several weeks until the population consisted mainly of fibroblastic cells. Clones were obtained from this population by limiting dilution and characterized for cadherin expression, morphology, and clonal integrity. Cadherin-negative clones were selected and renamed A431D cells to distinguish them from the parent A431 cells.

### Antibodies

Rat monoclonal (E9) and rabbit polyclonal antibodies against human E-cadherin (Wheelock et al., 1987) and rabbit pan-cadherin (Knudsen et al., 1995) and mouse monoclonal antibodies against  $\alpha$ -catenin (1G5) and  $\beta$ -catenin (5H10; Johnson et al., 1993), plakoglobin (15F11; Sacco et al., 1995), P-cadherin (6A9; Lewis et al., 1994), N-cadherin (13A9; Sacco et al., 1995), and desmoglein 2 (6D8; Wahl et al., 1996) have been previously described. The mouse monoclonal antidesmoplakin multi-epitope cocktail (dp) was purchased from American Research Products (Belmont, MA). The mouse monoclonal anticytokeratin antibody (CY90), which was made against A431 material, was purchased from Sigma Chemical Co.

### Molecular Constructs and Transfections

The human P-cadherin cDNA was a gift of Dr. Setsuo Hirohashi (National Cancer Center Research Institute, Tokyo, Japan). The entire P-cadherin cDNA was excised from pBR322 by EcoRI digestion and placed into the EcoRI site of pLKneo-1 expression vector (Hirt et al., 1992), which was a gift of Dr. Nicholas Fasel (University of Lausanne). Genes inserted into the pLK series of vectors are under control of the mouse mammary tumor virus promoter and are inducible by dexamethasone. The human E-cadherin cDNA was isolated from a JAR PR497 human gestational choriocarcinoma (Pattillo et al., 1971) cDNA library. The entire E-cadherin open reading frame was excised from pBluescript using HindIII and inserted into the HindIII site of pLKneo.

For cotransfection experiments, the neomycin gene in pLKneo was replaced with the puromycin gene from pBSpac $\Delta$ p (de la Luna and Ortín, 1992), a gift of Dr. Juan Ortín (Universidad Autónoma de Madrid, Madrid, Spain). This vector is referred to as pLKpac. The full-length human plakoglobin clone in pBluescript (HPG Ca2.1; Franke et al., 1989), a gift from Dr. Werner W. Franke (German Cancer Research Center, Heidelberg, Germany), was inserted into pLKpac. The chimera between E-cadherin and plakoglobin was constructed of (a) the entire extracellular and transmembrane domains of E-cadherin as well as the first 61 amino acids of its cytoplasmic domain, excluding the region shown to associate with  $\beta$ -catenin, (b) a nine-amino acid spacer, and (c) amino acids 19–745 of plakoglobin, which includes the domain that is necessary for association with  $\alpha$ -catenin (Sacco et al., 1995). The chimeric E-cadherin-plakoglobin cDNA was inserted into pLKpac.

Cell cultures were transfected using a calcium phosphate kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Colonies of G-418 or puromycin-resistant cells were isolated and screened for ex-

pression of the transfected gene(s) by immunoblot analysis. Positive clones were further examined by immunofluorescence microscopy, immunoblotting, and immunoprecipitation.

### Microscopy

Cells were plated in DME, 5% FCS with or without dexamethasone on glass coverslips and grown until almost confluent. Cells were fixed in 1% paraformaldehyde buffered with Hanks' balanced salt solution (Gibco Laboratories) and 10 mM Hepes followed by permeabilization in 100% cold methanol for 4 min at  $-20^{\circ}\text{C}$ . Coverslips were blocked for 1 h in PBS containing 10% goat serum and 0.1 M glycine and stained with primary antibodies for 1 h followed by FITC-conjugated anti-IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Fluorescence was detected with a microscope (model Axiophot; Carl Zeiss, Inc., Thornwood, NY) equipped with epifluorescence and filters appropriate for visualizing FITC, and cells were photographed using T-Max 3200 film.

### Electron Microscopy

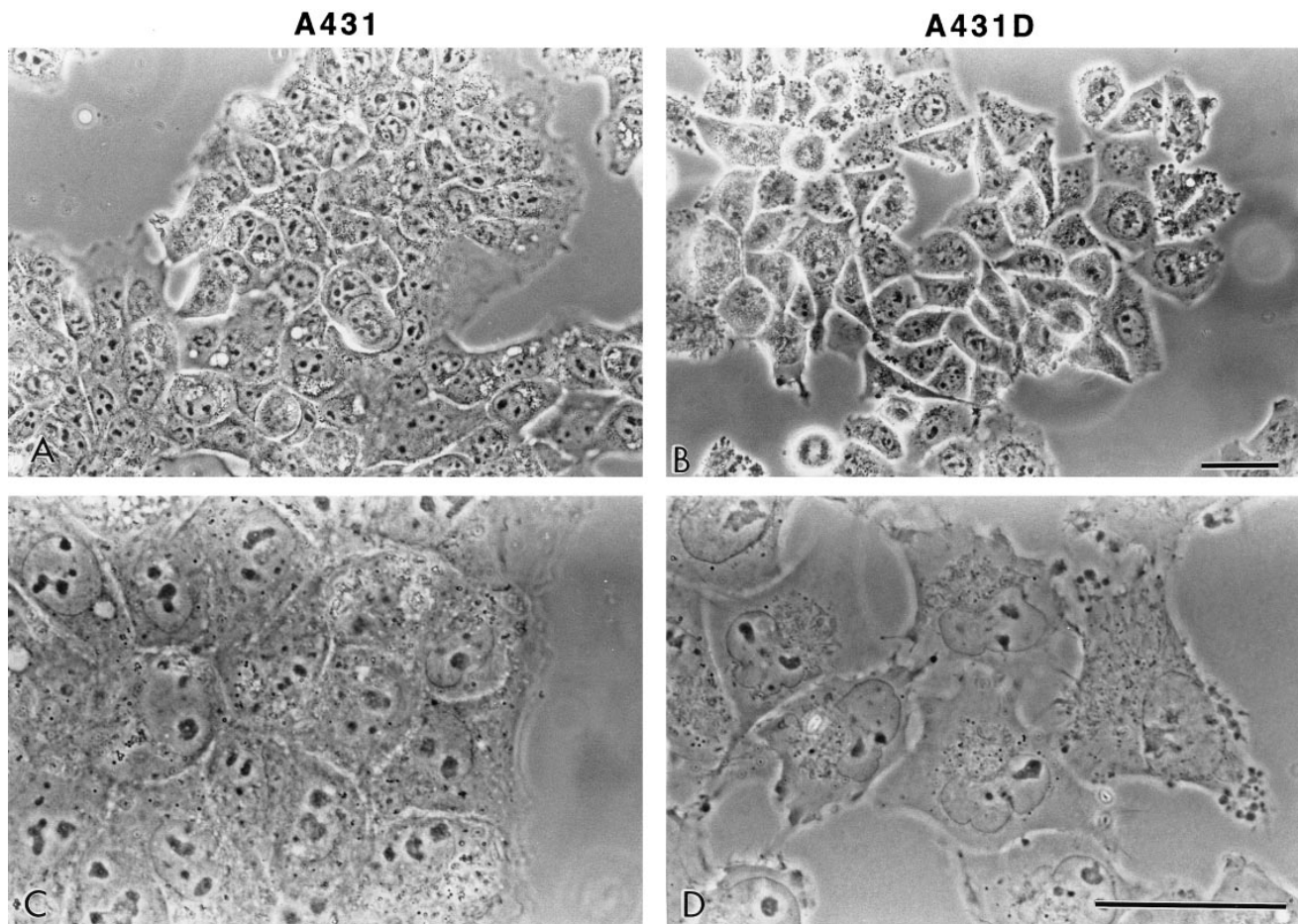
$2 \times 10^5$  cells were plated in DME, 5% FCS onto Falcon cyclopore membrane filters that inserted into 24-well plates (Becton-Dickinson Labware, Franklin Lakes, NJ). After incubation for 24–72 h, cultures were washed in protein-free DME, fixed overnight in 2% glutaraldehyde in 0.13 M sodium cacodylate buffer, pH 7.2, fixed in 2%  $\text{OsO}_4$  in 0.13 M cacodylate buffer, pH 7.2, dehydrated, infiltrated, and embedded in Epon. Thin sections were cut, stained with uranyl acetate and lead citrate, and examined on an electron microscope (model H7000; Hitachi Ltd., Tokyo, Japan).

### Protein Assays

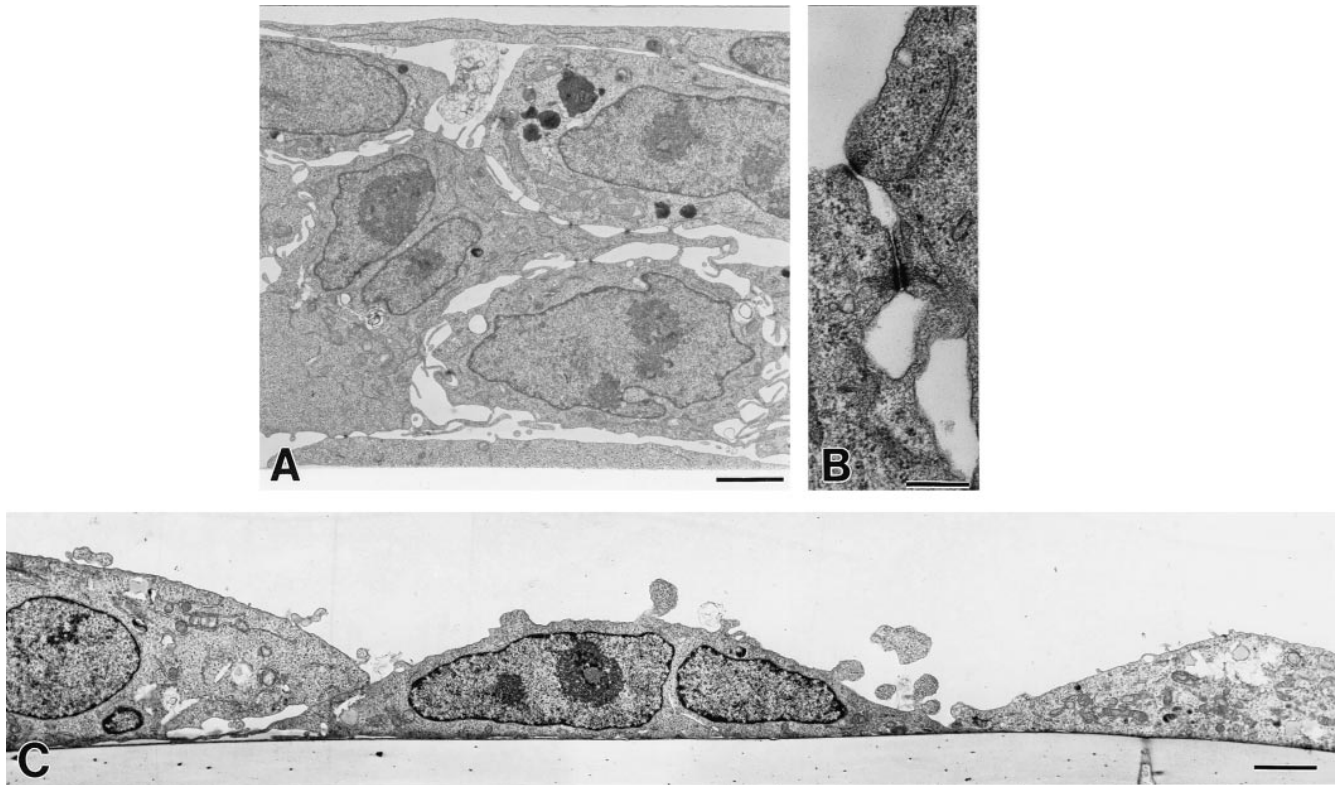
For the purpose of loading equal amounts of protein onto SDS-PAGE or for immunoprecipitation experiments, quantification was done using the BioRad Protein Assay reagent (Richmond, CA) according to the manufacturer's protocol.

### Immunoprecipitations

A431 cells were grown to confluence and either immediately extracted or metabolically labeled with  $[^{35}\text{S}][\text{Methionine/Cysteine}]$  (Translabel; ICN Biomedicals, Inc., Costa Mesa, CA) for 1 h followed by extraction as described (Lewis et al., 1994). Briefly, monolayers of cells were washed three times with PBS at room temperature, extracted at  $4^{\circ}\text{C}$  with 2 ml/75  $\text{cm}^2$  flask of 10 mM Tris acetate, pH 8.0, 0.5% NP-40 (BDH Chemicals Ltd., Poole, England), and 1 mM EDTA saturated with PMSF. The cells were scraped into this buffer, followed by vigorous agitation for 30 min on ice. Insoluble material was removed by centrifugation at 15,000  $g$  for 45 min.  $[^{35}\text{S}][\text{Methionine/Cysteine}]$ -labeled cell extracts were adjusted to 0.25 M NaCl and 0.3% BSA; 100  $\mu\text{l}$  extract was precleared by mixing with 50  $\mu\text{l}$  Sepharose 4B (Pharmacia LKB Biotechnology, Piscataway, NJ) at  $4^{\circ}\text{C}$  for 30 min. The supernatant was separated from the Sepharose by brief centrifugation. Unlabeled extracts were not precleared. Primary antibody was added to unlabeled or labeled extract and mixed at  $4^{\circ}\text{C}$  for 1 h. Antimouse or anti-rabbit IgG-Sepharose (Organon-Technica, Durham, NC) was added, and mixing continued for an additional 30 min. The Sepharose-bound immune complexes were washed five times with 50 mM Tris-HCl, pH 7.5, 0.5% NP-40, and 1 mM EDTA. Pellets of Sepharose-bound immune complex were boiled in Laemmli sample buffer (Laemmli, 1970)



**Figure 1.** A431D cells have fibroblast-like morphology. Living A431 cells (A and C) and A431D cells (B and D) were photographed using the 40 $\times$  objective (A and B) or the 100 $\times$  objective (C and D). Note the fibroblast-like morphology and the minimal cell-cell contact in the A431D cells. Note also the blebbing of the plasma membrane in the A431D cells. Bar, 30  $\mu\text{m}$ .



**Figure 2.** A431D cells grow as a monolayer without desmosomal contacts. A431 cells (A and B) and A431D cells (C) were plated on filters and processed for electron microscopy. A431 cells formed several layers with extensive desmosomal contacts. A431D cells grew as a monolayer and did not form desmosomes. Bars: (A and C) 2.5  $\mu\text{m}$ ; (B) 0.4  $\mu\text{m}$ .

and resolved on 7% SDS-PAGE as described (Lewis et al., 1994). For gels with labeled extracts  $^{14}\text{C}$ -labeled molecular mass markers (Gibco Laboratories) included myosin (205 kD), phosphorylase b (97 kD), BSA (66 kD), ovalbumin (43 kD), and carbonic anhydrase (30 kD). For gels with unlabeled extracts, molecular mass markers (Sigma Chemical Co.) included myosin (205 kD),  $\beta$ -galactosidase (116 kD), phosphorylase b (97 kD), BSA (66 kD), ovalbumin (43 kD), and carbonic anhydrase (30 kD).

Gels containing labeled extracts were enhanced (EN<sup>3</sup>HANCE, New England Nuclear, Boston, MA), dried, and autoradiographed with Kodak X-Omat AR film (Rochester, NY) at  $-70^{\circ}\text{C}$ . Gels containing unlabeled extracts were transferred electrophoretically to nitrocellulose and immunoblotted as described (Whelock et al., 1987) using primary antibodies followed by alkaline phosphatase-conjugated anti-IgG (Promega Corp., Madison, WI) and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates.

### Cell Fractionation

For some experiments, cells were fractionated into an aqueous-soluble fraction, an NP-40-soluble fraction, and an NP-40-insoluble fraction. Confluent 225  $\text{cm}^2$  flasks of cells were washed with PBS, scraped into 5 ml of 10 mM Tris acetate, pH 8.0, 1 mM EDTA (TE) saturated with PMSF at  $0^{\circ}\text{C}$ , and Dounce homogenized until all cells were broken but nuclei remained intact, as determined by phase microscopy. The extract was centrifuged at 15,000  $g$  for 45 min. The pellet was washed once with TE, extracted with 5 ml 0.5% NP-40 in TE, and centrifuged again at 15,000  $g$  for 45 min. The resulting pellet was solubilized in 5 ml of boiling Laemmli sample buffer (Laemmli, 1970).

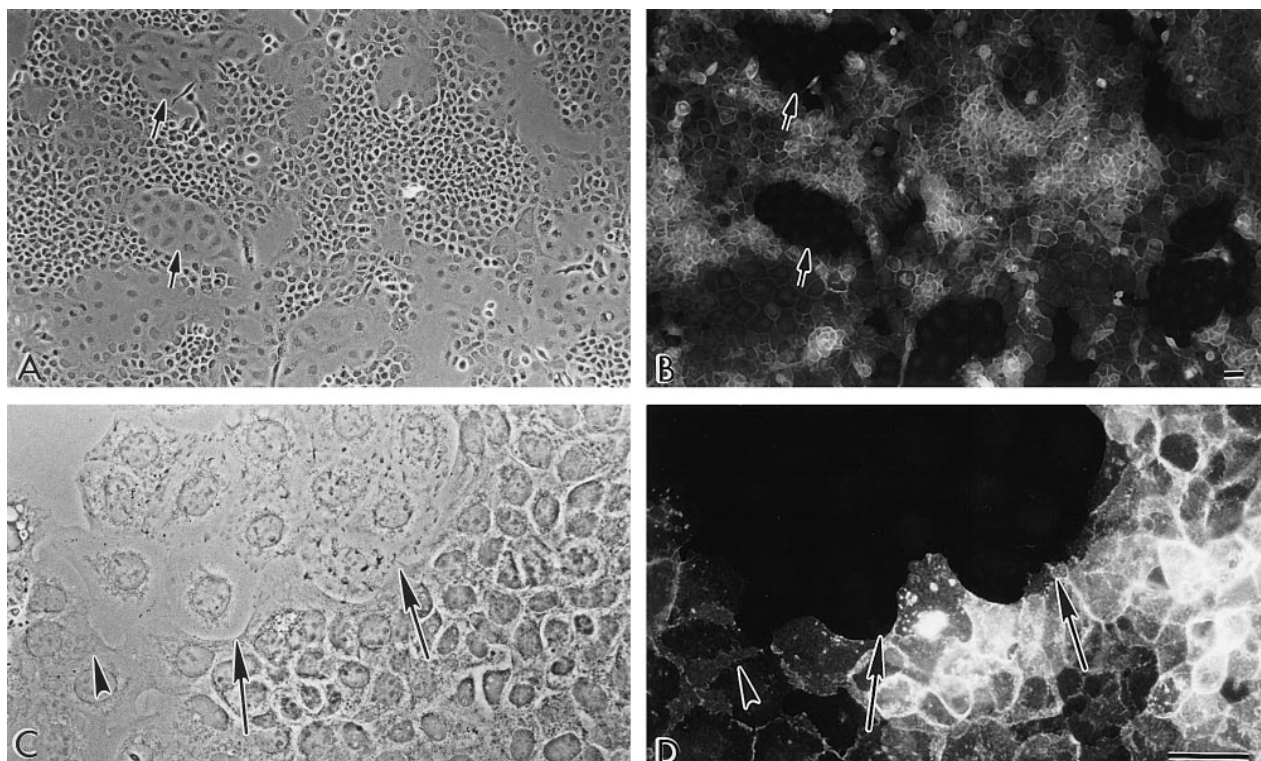
### Quantitative Analysis of Gels

Quantitative analysis of protein bands from immunoblots or labeled immunoprecipitation reactions was performed on a Macintosh Quadra 800 using the Plotting Macros of the NIH Image program (developed at the U.S. National Institutes of Health, Bethesda, MD, and acquired from the Internet by anonymous FTP from zippy.nimh.nih.gov).

## Results

### Treatment of A431 Cells with Dexamethasone Induces Dramatic Morphological Changes

Confluent monolayers of A431 cells had the cobblestone appearance that is typical of squamous epithelial cells. Treatment of A431 cultures with  $10^{-7}$  M dexamethasone resulted in the development of small islands of cells that showed distinct morphological changes from the parent cells, beginning at  $\sim 14$  d after addition of steroid. Specifically, the affected cells displayed a more fibroblastic morphology along with marked plasma membrane blebbing (Fig. 1). We found that we could enrich for the affected subpopulation by mild trypsinization, since these cells were detached much more readily by trypsin than the normal-appearing ones. Several rounds of such selective trypsinization, followed by further incubation and growth, led to the development of cultures that consisted almost entirely of the abnormal-appearing cells, which we renamed A431D cells to distinguish them from the parent A431 cell line. To characterize the A431D cells, several clones, all of which exhibited a similar phenotype, were isolated by limiting dilution. Aside from their fibroblastic morphology and membrane blebbing, another characteristic that distinguished the A431D cells from the parent A431 line was their growth pattern. Confluent A431 cultures exhibited several vertical layers with desmosomal attachments between layers; however, the A431D grew as a monolayer of cells without desmosomal attachments to



**Figure 3.** A431 cells show loss of E-cadherin staining as they convert to A431D cells. A431 cells were grown in  $10^{-7}$  M dexamethasone for 2 wk, transferred to glass coverslips, and processed for immunofluorescence. Phase microscopy (*A* and *C*) depicts the altered morphology of the cells as they are converted to A431D cells. Normal-looking A431 cells surround the islands of A431D cells in *A* and in the bottom right hand corner of *C*. Arrows in *A* and *B* point to islands of A431D cells. Arrows in *C* and *D* point to borders between the A431 parent cells and the A431D cells. Immunofluorescence microscopy shows cell border staining for E-cadherin (*B* and *D*). Note the absence of staining in the cells that have converted to A431D cells and the decreased staining in cells that appear to be converting to A431D cells (*arrowheads*). Bar, 30  $\mu$ m.

one another (Fig. 2). Although both types of cultures, when confluent, contained numerous floating cells, they exhibited very different properties. The floating cells of the parent A431 cultures were not viable, as evidenced by their inability to attach and proliferate when transferred to a new tissue culture plate; however, the floating cells of the A431D cultures rapidly reattached to a fresh culture plate and continued to grow (data not shown).

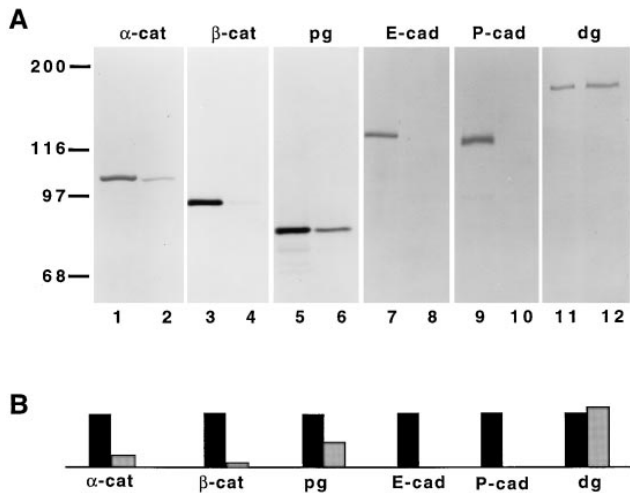
Both the morphological appearance as well as the unusual growth properties of the A431D cultures suggested to us that the adhesive properties of the cells were altered. We therefore compared the expression of adherens junction and desmosome components in the parent and derived cell lines.

#### ***Stable Expression of Most Adherens Junction Components Is Depressed in A431D Cells***

In the first series of experiments, we immunocytochemically stained A431 cultures that had been treated with dexamethasone for  $\sim 14$  d and therefore were comprised of areas with normal-appearing cells as well as foci with altered morphology. The areas of normal morphology consistently and intensely stained with antibodies to E-cadherin, P-cadherin,  $\alpha$ -catenin, and  $\beta$ -catenin, with concentration along the cell–cell borders, as expected for an epithelial cell line.

In marked contrast, the foci of altered morphology did not stain with antibodies to constituents of the adherens junction (Fig. 3). Cells that lacked expression of E- and P-cadherin initially appeared very flat and developed noticeable surface blebs with even fewer cell–cell contacts, as can be noted in Fig. 3, *A* and *C*. To analyze this population biochemically, we cloned out cadherin-negative cells from the population; all clones exhibited the more fibroblast-like appearance seen in Fig. 1, which appears to represent the final stage of the morphological changes induced by long-term culture in dexamethasone.

Several clones of A431D cells were then analyzed by immunoblotting and immunofluorescence for their levels of adherens junctional and desmosomal proteins. Since there was minimal variation, the results from one clone are presented. As shown in Fig. 4, there was no detectable E- or P-cadherin in the A431D cells, although comparable amounts of cell lysate protein from the A431 parent cells contained readily detectable amounts of these cadherins. When A431D cell extracts were immunoblotted with a monoclonal antibody against human N-cadherin or with a polyclonal anti-pan-cadherin antibody, the results were totally negative, indicating that the A431D cells did not express any classical cadherins (data not shown). Immunoblotting and immunofluorescence with antibodies to the catenins revealed that greatly decreased levels of both



**Figure 4.** A431D cells have decreased levels of cadherins and catenins. (A) A431 cells (odd numbered lanes) and A431D cells (even numbered lanes) were extracted with NP-40, and equal amounts of protein from each extract were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with various monoclonal antibodies. Lanes 1 and 2: anti- $\alpha$ -catenin ( $\alpha$ -cat); lanes 3 and 4: anti- $\beta$ -catenin ( $\beta$ -cat); lanes 5 and 6: anti-plakoglobin (*pg*); lanes 7 and 8: anti-E-cadherin (*E-cad*); lanes 9 and 10: anti-P-cadherin (*P-cad*); lanes 11 and 12: antidesmoglein (*dg*). Molecular weight markers are indicated. (B) Each band in A was quantified. The level of expression in A431 cells was assigned the value 1, and the level of expression in A431D is presented as a fraction of the level in A431.

$\alpha$ - and  $\beta$ -catenins were present in the A431D cells compared to the A431 parent line. To accurately compare A431D cells with A431 cells in immunofluorescence, timed exposures were taken (Fig. 5). Under conditions in which intense staining was found in A431 cells,  $\alpha$ -catenin,  $\beta$ -catenin, and plakoglobin were barely visible in A431D cells. When longer exposures were taken, however, all three catenins were detectable (Fig. 6 and not shown). In contrast, neither E-cadherin nor P-cadherin was detectable even after prolonged exposures (data not shown).

To compare the synthetic capacity of A431D and A431 cells for cadherins and catenins, both types of cultures were biosynthetically labeled with  $^{35}\text{S}$ [Methionine/Cysteine] for 1 h. Extracts were prepared from equal numbers of cells, subjected to immunoprecipitation with antibodies against each of these proteins, resolved with SDS-PAGE, and visualized with autoradiography. When immunoprecipitated with antibodies to either E- or P-cadherin, A431D extracts revealed no bands, although intense bands were present in the A431 parent extracts (data not shown). These data are consistent with the immunofluorescence and immunoblotting results, all of which demonstrate that A431D cells do not make detectable levels of E- or P-cadherin.

In contrast, when immunoprecipitation was performed with antibodies to the catenins, it became clear that A431D

cells synthesized close to normal levels of both  $\alpha$ - and  $\beta$ -catenin (Fig. 7 A). However, anticatenin antibodies did not coimmunoprecipitate other catenins or cadherins from the A431D extracts, although catenins, cadherins, and plakoglobin did coimmunoprecipitate in the A431 parent cell extracts. Hence, although A431D cells synthesize  $\alpha$ - and  $\beta$ -catenins, they do not form a complex with other cellular proteins. To examine the stability of the catenins, we performed pulse chase labeling experiments (Fig. 7 B). After a 1-h chase,  $\beta$ -catenin had almost completely disappeared in A431D cells. By contrast, in the parent A431 cells after a 2-h chase,  $\beta$ -catenin was still present at almost the same level as in the 0-h control. Interestingly,  $\alpha$ -catenin was stably expressed by A431D cells, at least for a 2-h chase period. The catenin biosynthetic labeling results, coupled with the evidence for reduced total levels of these proteins as observed in immunoblots and via immunofluorescence, suggest that  $\beta$ -catenin is expressed but then rapidly degraded in A431D cells. Given the total lack of E- and P-cadherins in the A431D cells, the instability of  $\beta$ -catenin is not surprising since in L cells, stable catenin expression has been shown to be dependent upon cadherin expression (Nagafuchi and Takeichi 1988; Ozawa et al., 1989). It was surprising, however, that  $\alpha$ -catenin was stably expressed. The level of expression, as shown by immunoblot analysis (see Fig. 4), was lower than in A431 cells, but the turnover rate was not as rapid as that for  $\beta$ -catenin. Consistent with  $\alpha$ - and  $\beta$ -catenins, plakoglobin was expressed at a lower level in A431D cells; its turnover rate in A431D cells and A431 cells appeared to be similar, presumably due to its association with desmosomal cadherins. Thus, our data concerning adherens junction proteins demonstrate that A431D cells have no detectable classical cadherins and decreased levels of  $\alpha$ -catenin,  $\beta$ -catenin, and plakoglobin.

#### Expression of Desmosomal Components Is Not Severely Affected in A431D Cells

Extracts of A431D or A431 cells were compared by immunoblot analysis for expression of the desmosomal cadherins (desmoglein and desmocollin). A431D cells showed approximately equal levels of desmocollin (data not shown) and desmoglein when compared with the parent A431 cells (Fig. 4).

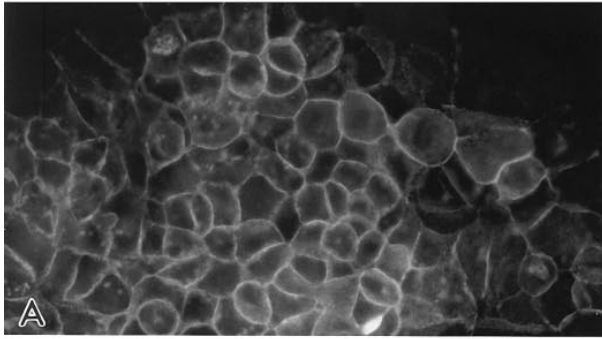
Immunofluorescence staining with antibodies against the desmosomal proteins similarly revealed the presence of these proteins in A431D cells (Figs. 5 and 8). However, the pattern of staining was distinct in the A431D and A431 cells. When A431 cells were examined at a higher magnification, it was clear that both desmoglein and desmoplakin were present in a punctate pattern along the cell-cell borders, indicative of desmosome organization (Figs. 8, A and C). In contrast, staining in the A431D cells was much more diffuse; desmoplakin was completely cytosolic (Fig. 8 D), and desmoglein appeared to be diffuse but with some indication of membrane staining. However, it was

**Figure 5.** Expression of adherens junction and desmosome proteins is abnormal in A431D cells. A431 cells (A, C, E, G, and I) or A431D cells (B, D, F, H, and J) were grown on glass coverslips and processed for immunofluorescence. (A and B) The localization of E-cadherin (*E-cad*); (C and D)  $\beta$ -catenin ( $\beta$ -cat); (E and F) plakoglobin (*pg*); (G and H) desmoglein (*dg*); (I and J) desmoplakin (*dp*). Timed exposures were taken to compare the levels of expression in A431D cells with that in A431 cells. Bar, 30  $\mu\text{m}$ .

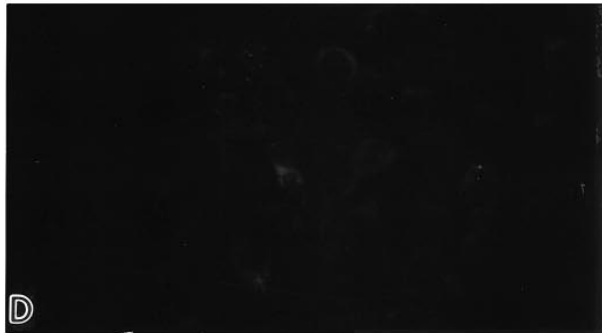
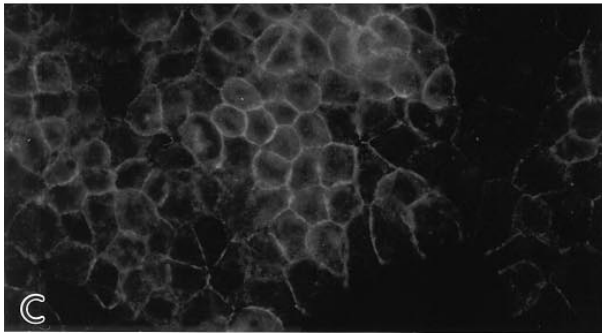


A431

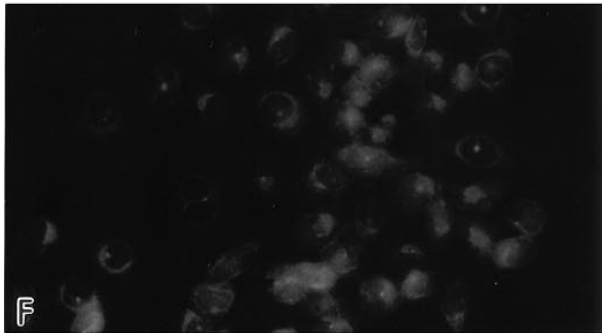
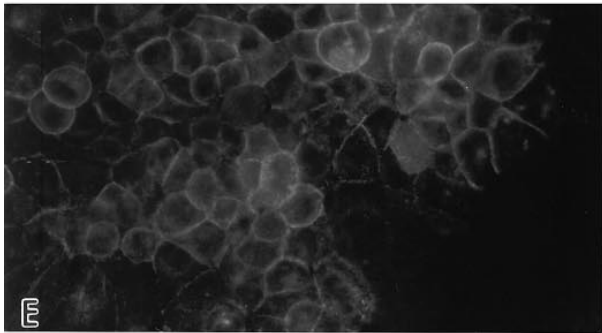
A431D



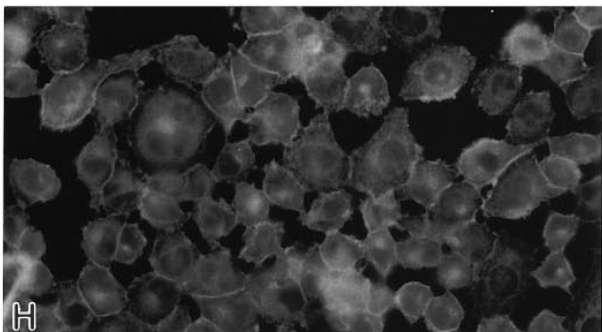
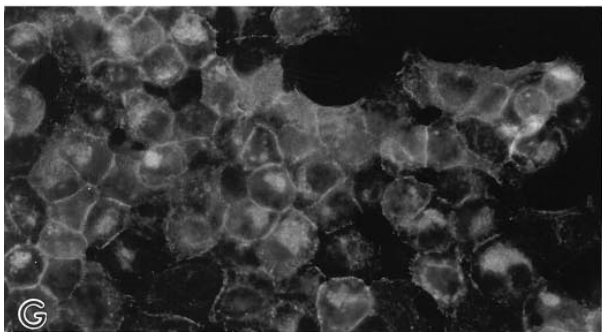
E-cad



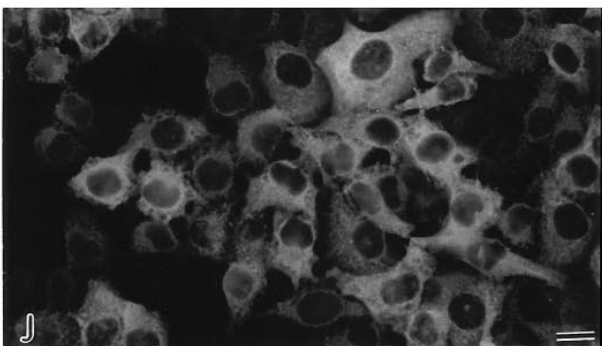
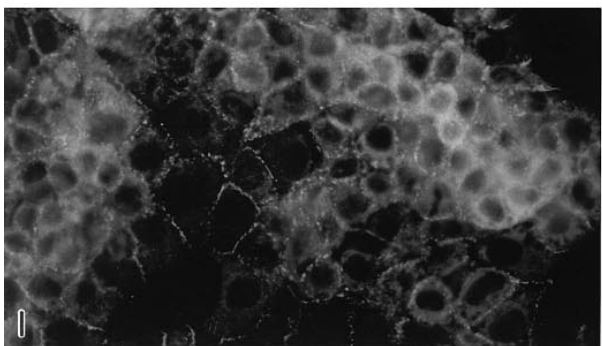
$\beta$ -cat



pg



dg



dp

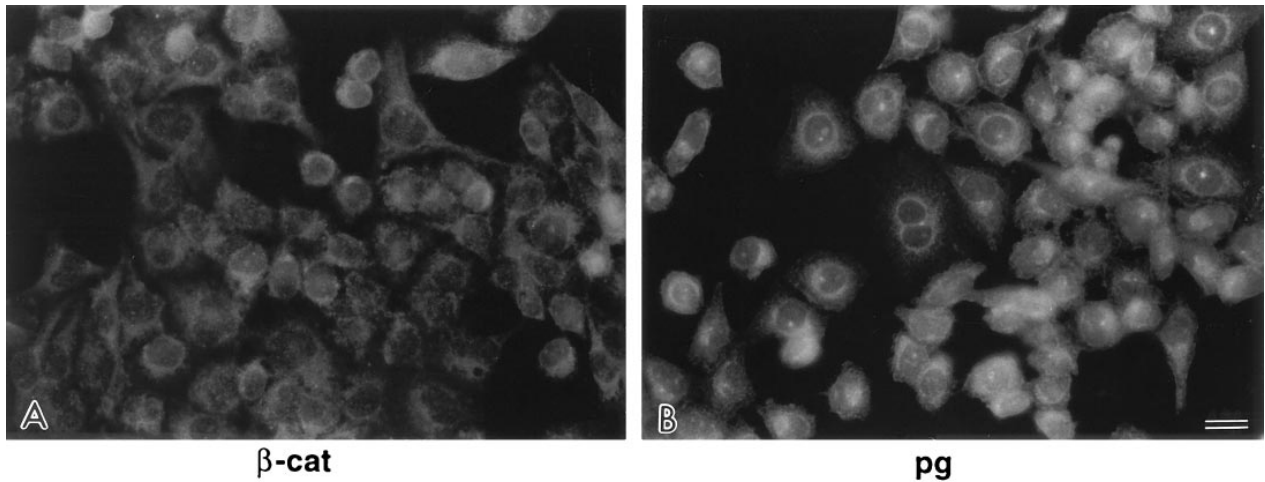


Figure 6.  $\beta$ -Catenin and plakoglobin are expressed at low levels by A431D cells. When automatic exposures were made of cells stained for  $\beta$ -catenin (A;  $\beta$ -cat) or plakoglobin (B; pg), each protein was detectable. Bar, 30  $\mu$ m.

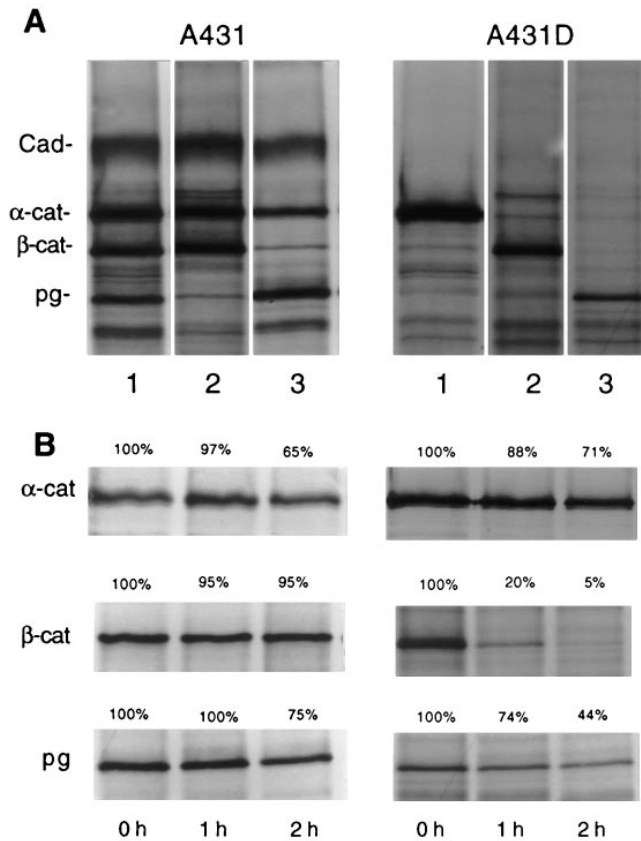


Figure 7. Catenins do not form normal complexes in A431D cells. (A) A431 cells and A431D cells were labeled with [ $^{35}$ S][Methionine/Cysteine] for 1 h and extracted with NP-40. Cell extracts were immunoprecipitated with monoclonal anti- $\alpha$ -catenin (lane 1), monoclonal anti- $\beta$ -catenin (lane 2), or monoclonal anti-plakoglobin (lane 3). E-cadherin, P-cadherin,  $\alpha$ -catenin,  $\beta$ -catenin, and plakoglobin coimmunoprecipitated with antibodies against  $\alpha$ -catenin (lane 1),  $\beta$ -catenin (lane 2), or plakoglobin (lane 3) from A431 cells. In contrast, only the catenin for which the immunoprecipitating antibody was specific was seen in the A431D reactions. (B) Cells were labeled for 1 h and chased for 1 or 2 h. They were then extracted with NP-40 and immunoprecipitated as

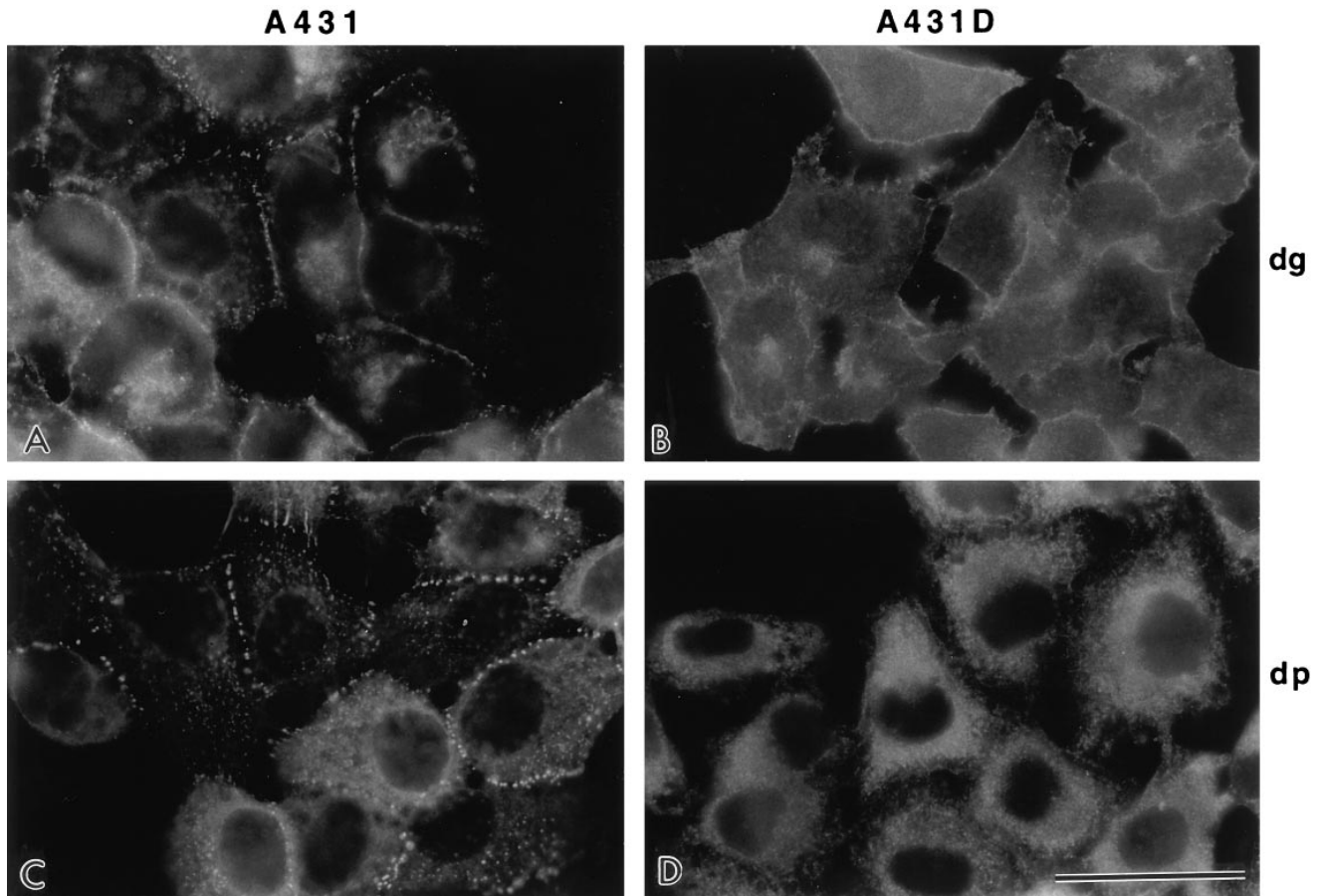
not present in a punctate pattern (Fig. 8 B). To rule out the possibility that A431D cells had lost the ability to either synthesize or properly organize keratin filaments, we compared staining patterns of A431 cells and A431D cells (Fig. 9). Cells were plated sparsely so that the A431 cells would have minimal cell-cell contact. Keratin filaments were expressed in A431D cells and were organized in a pattern similar, but not identical, to that seen in A431 cells. Thus, the data we have presented indicate that, although A431D cells synthesize the proteins necessary for desmosome formation, they are not able to organize these constituents into a functional structure (Figs. 2, 4, 5, and 8).

#### Transfection of E- or P-Cadherin into A431D Cells Restores Some but Not All Cadherin-related Functions

In previous studies using human keratinocytes and other epithelial cells, we and others have demonstrated that either E- or P-cadherin function is required for normal desmosome organization, as determined by redistribution of the desmosomal components to a punctate pattern along cell-cell borders and by ultrastructural analysis (Hodivala and Watt, 1994; Lewis et al., 1994; Amagai et al., 1995; Jensen et al., 1996). We therefore hypothesized that the lack of desmosomes in A431D cells was secondary to the loss of E- and P-cadherin expression. To test this hypothesis, we reexpressed a cadherin in the A431D cells by transfecting the entire cDNA for either E- or P-cadherin, using a plasmid that also conferred G418 resistance. Resistant colonies were screened for the expression of E- or P-cadherin by immunofluorescence and positive clones were se-

above.  $\alpha$ -Catenin and plakoglobin were stable in both A431 cells and A431D cells, whereas  $\beta$ -catenin was stable for 2 h in A431 cells but had almost completely disappeared by 1 h in A431D cells. The level of each catenin was quantified; expression at time 0 was set equal to 100%, and the remaining levels at each time point are expressed as a percentage of 0 time. The numbers are presented above each band. Cad, cadherin;  $\alpha$ -cat,  $\alpha$ -catenin;  $\beta$ -cat,  $\beta$ -catenin; pg, plakoglobin.





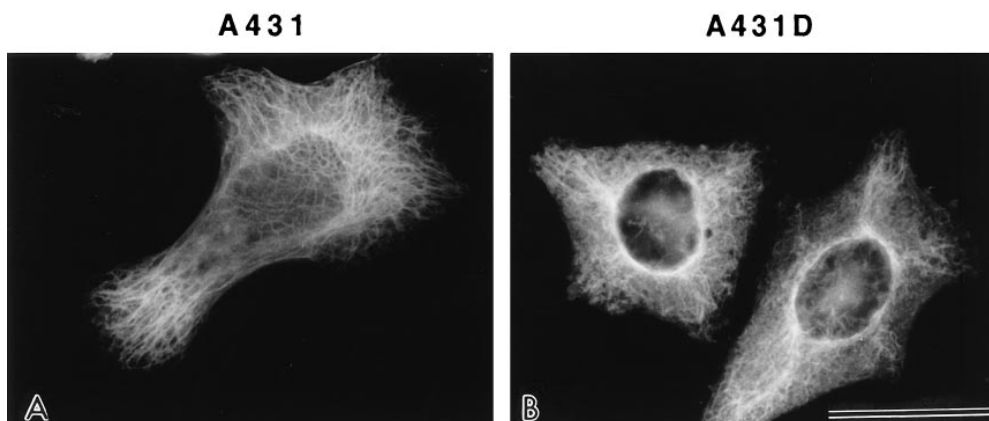
**Figure 8.** A431D cells show abnormal localization of desmosomal proteins. A431 cells (*A* and *C*) and A431D cells (*B* and *D*) were grown on glass coverslips, processed for immunofluorescence with antibodies against desmoglein (*A* and *B*; *dg*) or desmoplakin (*C* and *D*; *dp*), and photographed using a 100 $\times$  objective. Bar, 30  $\mu$ m.

lected. Clones of A431D cells transfected with E-cadherin (A431DE cells) or P-cadherin (A431DP) that expressed levels of cadherin nearly equivalent to that of the parent A431 cells were selected for further analysis (Fig. 10). As anticipated, A431DE cells expressed only E-cadherin and not P-cadherin, while A431DP cells expressed only P-cadherin and not E-cadherin. Thus, transfection of one cadherin cDNA did not lead to expression of the other cadherin by activation of the endogenous genes.

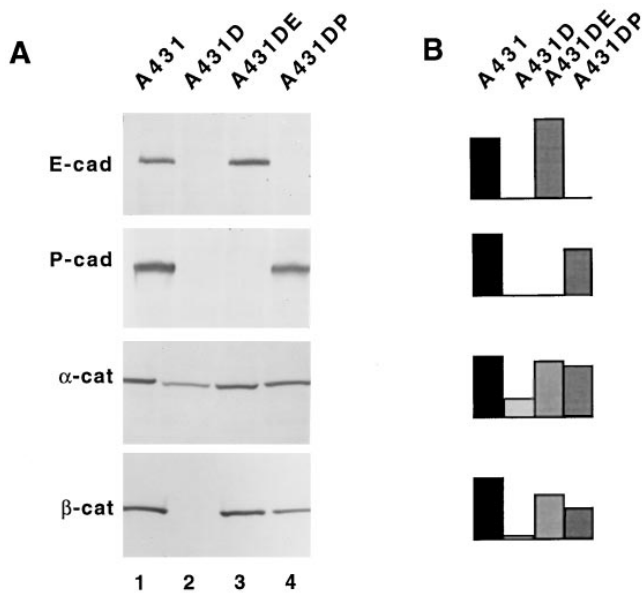
Stable expression of both  $\alpha$ - and  $\beta$ -catenin increased in

the A431D cells transfected with either E- or P-cadherin (Fig. 10). These results confirm our hypothesis that the lower levels of  $\alpha$ - and  $\beta$ -catenin in A431D cells were due to instability because of a lack of association with cadherin rather than to a direct effect of dexamethasone treatment.

Immunofluorescence localization of either E-cadherin in the A431DE cells (Fig. 11 *A*) or P-cadherin in the A431DP cells (data not shown) showed that the cadherin was found at cell-cell borders and was not diffusely distributed in the cytoplasm, suggesting that the cadherin was



**Figure 9.** The pattern of keratin staining is similar in A431 cells and A431D cells. A431 cells (*A*) or A431D cells (*B*) were grown on glass coverslips and processed for immunofluorescence with antibodies against keratin. Bar, 30  $\mu$ m.

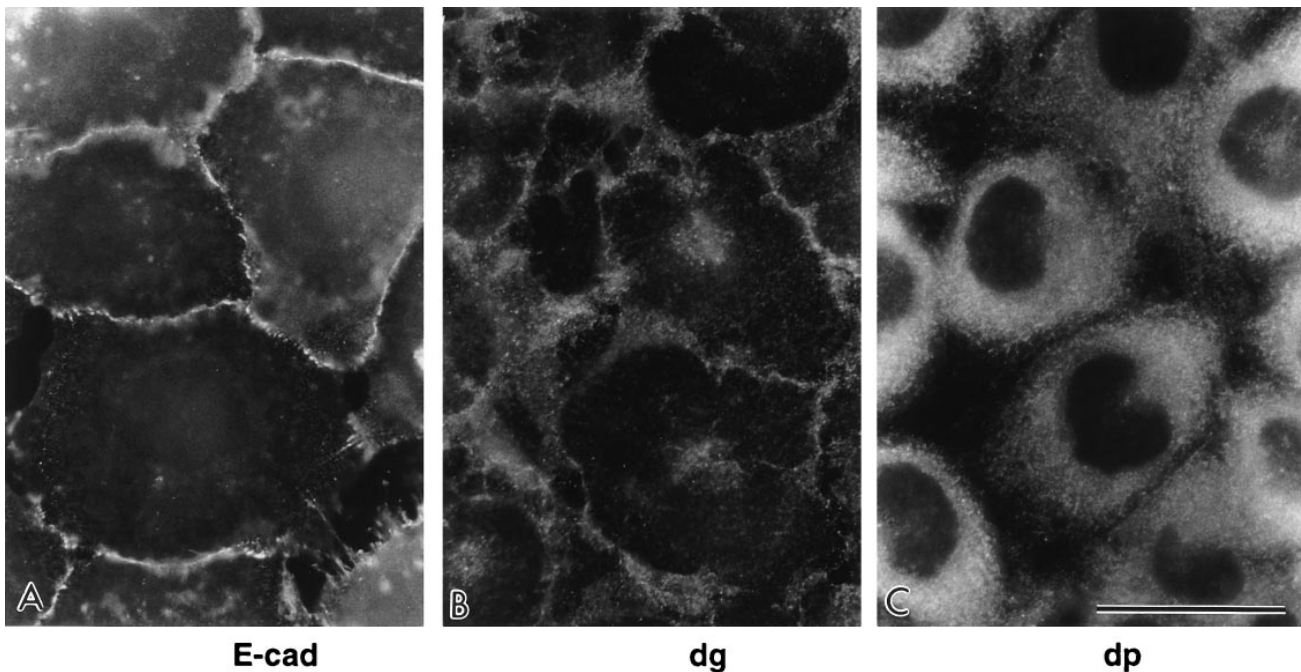


**Figure 10.** Transfection of E- or P-cadherin into A431D cells stabilizes the expression of  $\alpha$ - and  $\beta$ -catenin. (A) A431 cells (lane 1), A431D cells (lane 2), A431D cells transfected with E-cadherin (A431DE; lane 3), and A431D cells transfected with P-cadherin (A431DP; lane 4) were extracted with NP-40 and equal amounts of protein from each extract were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibodies against E-cadherin (*E-Cad*), P-cadherin (*P-cad*),  $\alpha$ -catenin ( *$\alpha$ -cat*), or  $\beta$ -catenin ( *$\beta$ -cat*). (B) Each band in A was quantified. The level of expression in A431 cells was assigned the value 1, and the levels of expression in the other cell lines are presented as fractions of the level in A431.

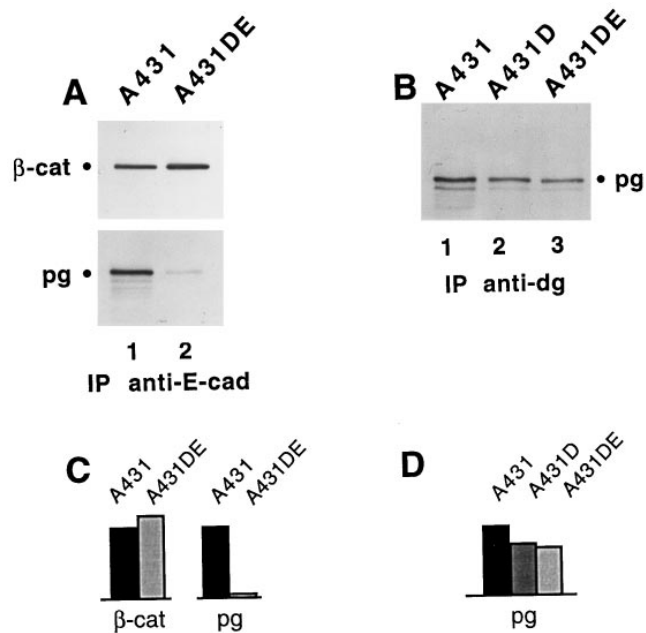
organized into junctional structures. The desmosomal protein desmoglein was also localized at cell-cell borders, but it did not exhibit the punctate pattern that is indicative of desmosome formation (compare Fig. 11 B with 8 A). Furthermore, desmoplakin remained in a diffuse cytoplasmic pattern (Fig. 11 C) similar to that of A431D cells (Fig. 8 D). These data indicate that, despite the expression and cell-cell border localization of the classical cadherin in these cells, the desmosomal proteins were not being organized into punctate structures indicative of desmosomes. Throughout the remainder of this paper, only the A431DE cells will be discussed, although identical experiments were carried out for the A431DP cells, and similar results were obtained.

To evaluate in another way the functionality of the transfected cadherin, we tested its ability to form complexes with the cellular catenins. Whole cell extracts of the A431DE cells were immunoprecipitated with anti-E-cadherin antibodies, followed by immunoblot analysis to look for the presence of coimmunoprecipitated  $\beta$ -catenin or plakoglobin. As shown in Fig. 12 A,  $\beta$ -catenin coimmunoprecipitated with E-cadherin to an equivalent extent in A431 and A431DE cells. In contrast, only very small amounts of plakoglobin coimmunoprecipitated with the transfected E-cadherin when compared with that normally seen in A431 cells (Fig. 12 A).

To test the possibility that plakoglobin expressed by the A431DE cells was altered such that it could not associate with any cadherins, we immunoprecipitated desmoglein from A431, A431D, and A431DE cells and then compared the levels of plakoglobin coimmunoprecipitated with this cadherin in each cell line (Fig. 12 B). In all three cell lines,



**Figure 11.** A431D cells transfected with E-cadherin do not regain the ability to organize desmosomal proteins at cell-cell borders. A431D cells transfected with E-cadherin (A431DE) were grown on glass coverslips and processed for immunofluorescence. (A) The localization of E-cadherin (*E-cad*); (B) the localization of desmoglein (*dg*); and (C) the localization of desmoplakin (*dp*). Bar, 30  $\mu$ m.



**Figure 12.** Plakoglobin is not associated with E-cadherin in A431DE cells. (A) A431 cells (lane 1) or A431D cells transfected with E-cadherin (A431DE; lane 2) were extracted with NP-40 and equal amounts of protein from each extract were immunoprecipitated (IP) with antibodies against E-cadherin (*anti-E-cad*). The immunoprecipitated proteins were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibodies against  $\beta$ -catenin ( $\beta$ -cat) or plakoglobin (pg).  $\beta$ -Catenin coimmunoprecipitated with the transfected E-cadherin, whereas plakoglobin did not. (B) A431 cells (lane 1), A431D cells (lane 2), or A431DE (lane 3) were extracted with NP-40, and equal amounts of protein from each extract were immunoprecipitated with antibodies against desmoglein (*dg*). The immunoprecipitation reactions were resolved by 7% SDS-PAGE, transblotted to nitrocellulose, and probed with monoclonal antibodies against plakoglobin. (C) Each band in A was quantified. The amount of  $\beta$ -catenin or plakoglobin that was associated with E-cadherin in A431 cells was assigned the value 1 and the amount in A431DE cells is presented as a fraction of that in A431. (D) Each band in B was quantified. The amount of plakoglobin that was associated with desmoglein in A431 cells was assigned the value 1, and the amounts in the other cell lines are presented as fractions of that in A431.

plakoglobin was found to be complexed with desmoglein to a similar (although not identical) extent. Similarly, plakoglobin was also associated with desmocollin in all the tested cell lines (data not shown). The domain of plakoglobin that associates with the classical cadherins overlaps the domain that associates with the desmosomal cadherins (Chitaev et al., 1996; Wahl et al., 1996; Witcher et al., 1996). Thus, the inherent ability of plakoglobin to associate with desmosomal (and presumably classical) cadherins did not seem to be completely disrupted in A431D cells.

#### **Plakoglobin Association with E-Cadherin Is Necessary for Desmosome Organization**

As described in the previous section, transfected E-cadherin in A431DE cells appeared in many ways to be functional in that it associated with  $\alpha$ - and  $\beta$ -catenins and was

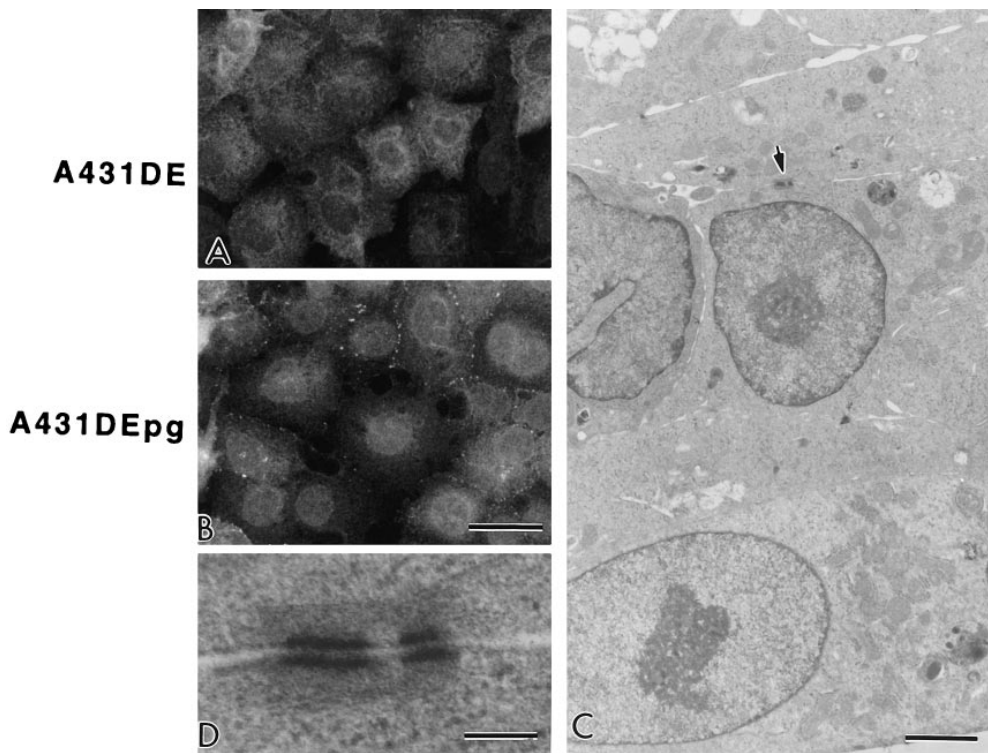
localized along cell–cell borders. Surprisingly, it did not complex with cellular plakoglobin, and it apparently could not mediate desmosome organization. Based on these observations, we hypothesized that the inability of the A431DE cells to form desmosomes might be related to a lack of E-cadherin–plakoglobin interaction. To address this hypothesis, we attempted to increase the amount of E-cadherin–plakoglobin complex within the A431DE cells by overexpressing plakoglobin. A431D cells were therefore cotransfected with full-length plakoglobin cDNA as well as E-cadherin cDNA, and coexpressing clones were isolated (A431DEpg cells).

To determine whether restoration of the plakoglobin level led to restoration of the ability to form desmosomes, we performed immunofluorescence and electron microscopic analyses. Staining of A431DEpg cells with antibody to desmoplakin revealed a punctate pattern along the cell–cell borders (Fig. 13 B), strongly suggesting formation of desmosomes. This was confirmed by ultrastructural analysis which demonstrated the presence of desmosomes in A431DEpg cells (Fig. 13, C and D).

To demonstrate that we had in fact increased the level of classical cadherin–plakoglobin complex in the double transfectants, we semiquantified the relative levels of plakoglobin in different compartments of the parent and transfected cell lines using immunoblots. We prepared three sequential cell fractions: (a) an aqueous soluble fraction prepared by homogenizing the cells in the absence of detergent, (b) a nonionic detergent-soluble fraction prepared by NP-40 extraction of the pellet recovered from the first step, and (c) an insoluble or cytoskeletal fraction prepared by solubilizing the pellet from the second step in SDS. Fraction b was further fractionated into plakoglobin that was associated with classical cadherins and plakoglobin that was associated with desmosomal cadherins by exhaustive immunoprecipitation. As shown in Fig. 14, the amount of plakoglobin in each fraction was very similar in A431 and A431DEpg cells. Furthermore, the amount of plakoglobin complexed to cadherins (in the NP-40 soluble fraction) was also very similar in the two cell lines. All of these data thus demonstrate that the amount and distribution of plakoglobin in the A431DEpg transfectant is very close to that observed in the parent A431 cells.

An important control for this experiment was the demonstration that transfection of plakoglobin alone into A431D cells was not sufficient for desmosomal organization. This is demonstrated in Fig. 15, which shows the staining pattern for desmoplakin in A431D cells that were overexpressing plakoglobin in the absence of a classical cadherin. Desmoplakin was localized throughout the cytoplasm in a diffuse pattern; no desmoplakin was detected at the cell–cell borders. Even though these clones were expressing as much plakoglobin as the parent A431 cells (as demonstrated by immunoblotting, not shown), they could not organize desmoplakin in the absence of a classical cadherin.

The most straightforward interpretation of all these data is that desmosome organization requires a sufficient level of plakoglobin to permit complex formation both with desmosomal cadherins and with classical cadherins. Hence, we hypothesize that the complex between classical cadherins and plakoglobin has signal-transducing capacity that initiates desmosome organization.



**Figure 13.** Cotransfection of A431D cells with E-cadherin and plakoglobin results in formation of desmosomes. A431D cells transfected with E-cadherin (A431DE; A) or cotransfected with E-cadherin and plakoglobin (A431DEpg; B) were grown on glass coverslips and processed for immunofluorescence with antibodies against desmoplakin. A punctate pattern of desmoplakin staining indicative of desmosome organization was observed in the A431DEpg cells but not in the A431DE cells. A431DEpg cells were plated on filters and processed for electron microscopy (C and D). The cells grew in several layers and formed desmosomal contacts. The arrow in C points to a desmosome shown at higher magnification in D. Bars: (A) 30  $\mu\text{m}$ ; (C) 2  $\mu\text{m}$ ; (D) 0.2  $\mu\text{m}$ .

#### ***A Plakoglobin–E-Cadherin Chimera Can Restore Desmosome Organization***

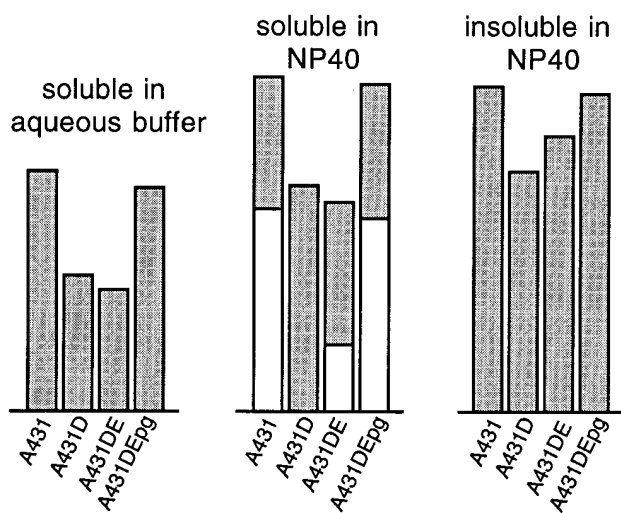
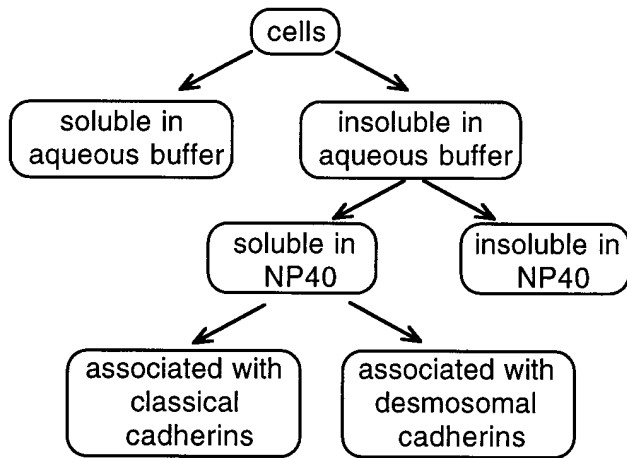
To test further our hypothesis that a plakoglobin–classical cadherin complex is required to initiate desmosome organization, we constructed cDNA for an E-cadherin–plakoglobin chimeric molecule (Fig. 16 B) consisting of: (a) the entire extracellular and transmembrane domains of E-cadherin as well as the first 61 amino acids of its cytoplasmic domain, excluding the region shown to associate with  $\beta$ -catenin (Stappert and Kemler, 1994), (b) a 9–amino acid spacer, and (c) amino acids 19–745 of plakoglobin, which includes the domain that is necessary for association with  $\alpha$ -catenin (Sacco et al., 1995). This chimeric cDNA was transfected into A431D cells. Clones expressing the chimeric protein (A431DchiE/pg) were verified by immunoblotting with anti–E-cadherin and antiplakoglobin antibodies, both of which reacted with a protein at 150 kD, the expected molecular mass (not shown). Immunoprecipitations of the chimeric protein with anti–E-cadherin antibodies showed that it was capable of associating with  $\alpha$ -catenin, as expected (data not shown). Localization of desmoplakin in the A431DchiE/pg cells revealed a punctate pattern concentrated along the cell–cell borders, highly indicative of desmosome organization and indistinguishable from the parent A431 cells (Fig. 16).

Thus, our data with the chimeric transfectants verify and extend our findings with the cotransfected lines expressing both E-cadherin and plakoglobin. An association between a classical cadherin and plakoglobin appears to be essential for the formation of desmosomes. Plakoglobin appears to be a central molecule in desmosomal organization, not only because of its presumed structural role but also because of a regulatory function in conjunction with the classical cadherins.

#### ***Discussion***

The goal of these studies was to further our understanding of how classical cadherins (e.g., E- and P-cadherins) regulate the organization of desmosomes. In previous studies using normal human keratinocytes incubated with function-perturbing antibodies against the cadherins, we and others have shown that either E- or P-cadherin must be functional in order for these cells to form desmosomes normally (Hodivala and Watt, 1994; Lewis et al., 1994; Amagai et al., 1995; Jensen et al., 1996). Similar observations have been made with other epithelial cells, but the mechanism involved in cadherin-regulated formation of desmosomes has been difficult to address. Our approach has been to generate a cell line that expresses the components of the desmosome but does not express a classical cadherin. We created this cell line by treating A431 cells with dexamethasone for an extended period of time. Upon treatment with  $10^{-7}$  M dexamethasone for about 2 wk, A431 cultures formed foci with a distinct morphology; examination of these foci revealed a lack of both E- and P-cadherin. Upon further analysis, it became clear that the affected cells, named A431D cells, were still synthesizing the catenins, although they were not stable in the cell, probably because of lack of interaction with a cadherin. Interestingly, the A431D cells expressed all of the desmosomal components examined at close to normal levels, but they did not form desmosomes.

In the present study, we have not addressed the mechanism by which A431 cells ceased to express the cadherins when treated with dexamethasone. The switch-off of the cadherins appeared to take place over several weeks and did not involve every cell. However, the cadherin-negative cells could be easily recovered from the culture because they



**Figure 14.** Relative amount and distribution of plakoglobin are similar in A431 and A431DEPg cells. Cells were fractionated into soluble (no detergent), NP-40-soluble, and NP-40-insoluble fractions. Equal volumes of each fraction was resolved by SDS-PAGE, immunoblotted for plakoglobin, and quantified. The NP-40-soluble fraction was further fractionated into E- and P-cadherin-associated plakoglobin and into desmoglein- and desmocollin-associated plakoglobin by exhaustive immunoprecipitation. Each fraction is represented graphically. The white insets in the NP-40-soluble graph represent the plakoglobin that was associated with E-cadherin + P-cadherin. The remainder was associated with desmoglein + desmocollin.

floated in the medium when the culture became confluent. These floating, cadherin-negative cells could be readily propagated since they would replat and divide when added to a fresh culture dish. We are still attempting to determine the mechanism by which cadherins are turned off; we suspect it involves alterations in the activity of the cadherin promoter. We also believe that the mechanism has specificity for A431 cells, as we attempted, without success, to replicate the dexamethasone effect in other squamous epithelial cell lines.

Regardless of the mechanism by which the A431D cells were generated, they are a useful experimental tool for

evaluation of the molecular interactions that are required for intercellular junction organization. Our present studies with these cells suggest that plakoglobin is a central regulatory molecule. A431D cells express approximately half the plakoglobin of the parent A431 cell line. When A431D cells are transfected with E-cadherin, the plakoglobin level remains approximately the same, and most remains associated with desmosomal cadherins. Very little plakoglobin is associated with the transfected E-cadherin, even though  $\alpha$ - and  $\beta$ -catenins readily form complexes with the transfected cadherin. Recently, Chitaev et al. (1996) showed that the binding affinity of plakoglobin for the desmosomal cadherins is five times stronger than for the classical cadherins, indicating that the preferential association we observe could be explained on the basis of stronger interactions at the protein level.

Our findings with the A431D cells strongly indicate that expression of desmosomal components and interaction of plakoglobin with desmosomal cadherins are not sufficient for desmosome organization. At least two additional conditions must be met if desmosome formation is to proceed: (a) The cells must express a classical cadherin; and (b) there must be sufficient plakoglobin to permit association not only with desmosomal cadherins, but also with the classical cadherins. It has previously been shown that plakoglobin is not required for organization of an adherens junction (Sacco et al., 1995), since  $\beta$ -catenin can be used in its stead. However, the present results indicate that adherens junctions containing only  $\beta$ -catenin cannot direct subsequent desmosome organization; rather, plakoglobin interaction with a classical cadherin, probably in the context of an adherens junction, is necessary to organize the desmosome components.

Ruiz et al. (1996) recently showed that plakoglobin knock-out mice could assemble desmosomes in the squamous epithelial cells of the skin but not in the muscle cells of the heart. These authors postulated that other armadillo family members, in particular plakophilin 1, which is expressed in the skin but not in the heart, may be able to substitute for plakoglobin. Consistent with this idea, our line of A431 cells does not express plakophilin 1.

The assembly of structures like the desmosome and the adherens junction is indeed complex. It is interesting that one protein, plakoglobin, is found in both structures, while the remaining components are restricted to one structure or the other. Bornslaeger et al. (1996) have presented data suggesting that desmoplakin plays a role in segregating adherens junction and desmosomal proteins. In their studies, cells expressing a mutant, dominant-negative desmoplakin were not able to restrict adherens junction components from regions where desmosomal cadherins were localized. These authors suggested that an interaction between plakoglobin and desmoplakin (although no direct interaction has been demonstrated) may play a role in organizing the desmosomal and adherens junctional components into their separate structures.

Plakoglobin and  $\beta$ -catenin are members of the armadillo family of proteins. Recent studies (for review see Peifer, 1995) have suggested that armadillo family members can act as signaling molecules in addition to their structural role in the adherens junction. Our results suggest that one signaling function for plakoglobin is to direct desmo-

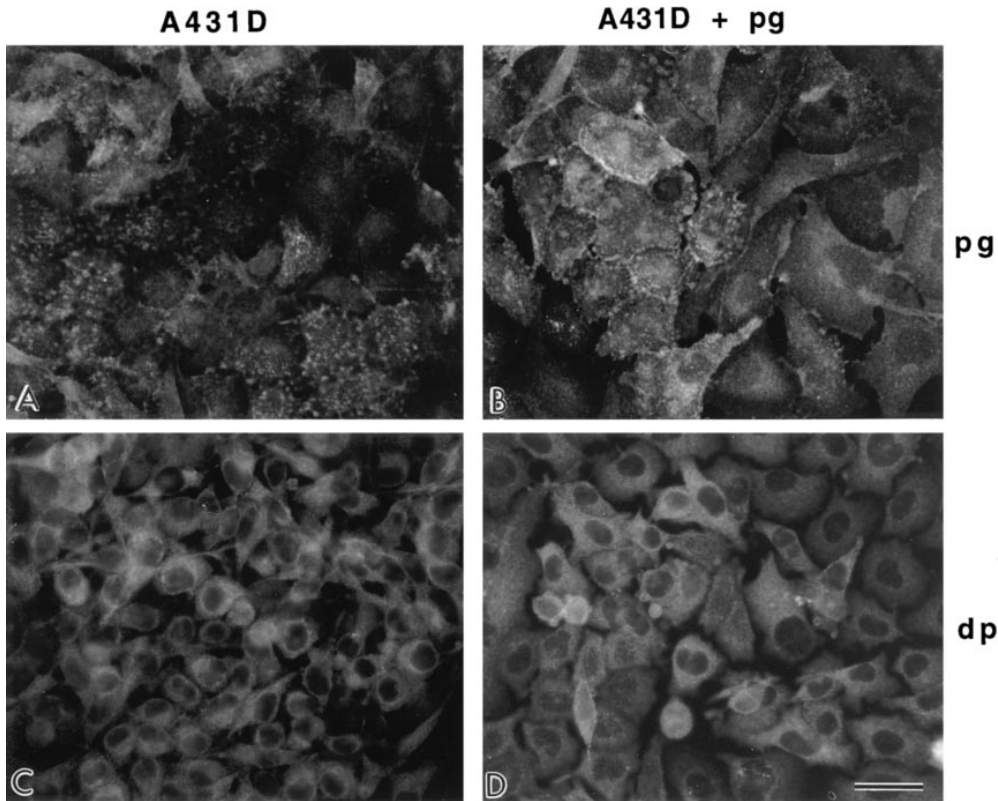


Figure 15. Overexpression of plakoglobin alone does not result in organization of desmoplakin at cell-cell borders. A431D cells (A and C) and A431D cells transfected with plakoglobin (pg; B and D) were grown on glass coverslips and processed for immunofluorescence with antibodies against plakoglobin (A and C) or desmoplakin (dp; B and D). Bar, 30  $\mu$ m.

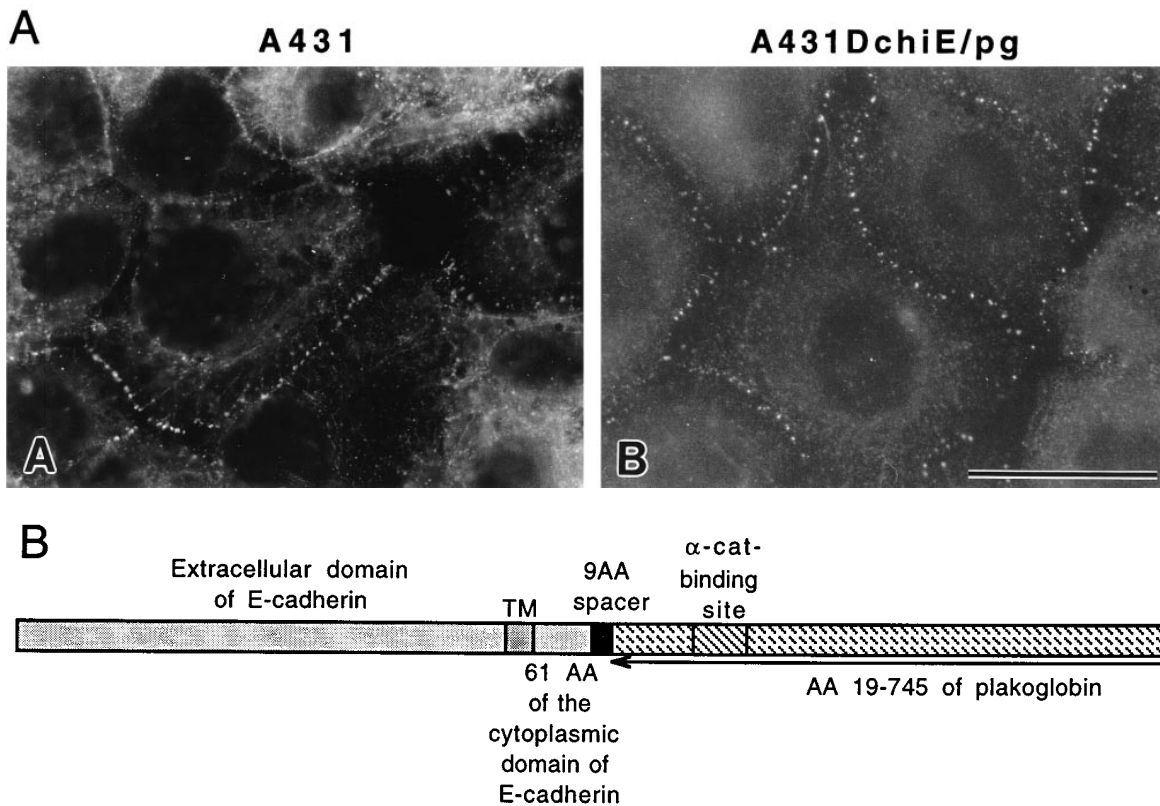


Figure 16. Transfection of an E-cadherin-plakoglobin chimeric protein restores the ability of A431D cells to organize desmoplakin at cell-cell borders. (A) A431 cells (A) and A431D cells transfected with the chimera (A431DchiE/pg; B) were grown on glass coverslips and processed for immunofluorescence with antibodies against desmoplakin. (B) The chimeric molecule consisted of the entire extracellular and transmembrane (TM) domains of E-cadherin as well as the first 61 amino acids of its cytoplasmic domain, excluding the region shown to associate with  $\beta$ -catenin, a nine-amino acid spacer, and amino acids 19-745 of plakoglobin, which includes the domain that is necessary for association with  $\alpha$ -catenin. Bar, 15  $\mu$ m.



some organization. It is interesting that plakoglobin (which is a component of both adherens junctions and desmosomes) can perform this function, but  $\beta$ -catenin (which is restricted to adherens junctions) cannot. One theoretical explanation for this divergence of function between plakoglobin and  $\beta$ -catenin is that formation of a desmosome involves exchange of plakoglobin molecules between the classical cadherin and the desmosomal cadherin, an interaction not possible for  $\beta$ -catenin. However, our studies using A431D cells transfected with a cadherin-plakoglobin chimera make this explanation highly unlikely since these cells could organize desmosomes even though E-cadherin was associated permanently with plakoglobin. With ongoing studies in our laboratory using chimeric molecules containing  $\beta$ -catenin and plakoglobin domains, we are attempting to understand the differences between these two proteins.

Armadillo family members are components of the wingless or wnt-signaling pathway (for review see Orsulic and Peifer, 1996). Karnovsky and Klymkowsky (1995) showed that overexpression of plakoglobin in *Xenopus* embryos mimicked the effect of overexpression of wnt. Expression of exogenous wnt 1 by some tissue culture cells results in increased cell-cell adhesion (Bradley et al., 1993; Hinck et al., 1994). It is possible that overexpression of plakoglobin in the A431D cells mimics a wnt signal, which results in increased cell-cell adhesion. We have cocultured A431DE cells with wnt 1-expressing Rat 2 cells (kindly provided by Dr. Anthony Brown; Jue et al., 1992) and have not observed any effect on the ability of the cells to organize desmosomal components. This does not, however, rule out a role for the wnt family in desmosomal organization because our cells may not express the appropriate receptor for wnt 1. The wnt family is large (Nusse and Varmus, 1992), and the recently identified family of wnt receptors (frizzled; Bhanot et al., 1996) is also very large (Wang et al., 1996). Further studies along these lines require identification of the specific wnt receptors expressed by A431D cells as well as the functional pairing of specific wnt family members with the appropriate frizzled receptors.

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