The Vertebrate Adhesive Junction Proteins β-catenin and Plakoglobin and the *Drosophila* Segment Polarity Gene *armadillo* Form a Multigene Family with Similar Properties

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Abstract. Three proteins identified by quite different criteria in three different systems, the *Drosophila* segment polarity gene *armadillo*, the human desmosomal protein plakoglobin, and the *Xenopus* E-cadherin–associated protein β-catenin, share amino acid sequence similarity. These findings raise questions about the relationship among the three molecules and their roles in different cell–cell adhesive junctions. We have found that antibodies against the *Drosophila* segment polarity gene *armadillo* cross react with a conserved vertebrate protein. This protein is membrane associated, probably via its interaction with a cadherin-like molecule. This cross-reacting protein is the cadherin-associated protein β-catenin. Using anti-*armadillo* and antiplakoglobin antibodies, it was shown that β-catenin and plakoglobin are distinct molecules, which can coexist in the same cell type. Plakoglobin interacts with the desmosomal glycoprotein desmoglein I, and weakly with E-cadherin. Although β-catenin interacts tightly with E-cadherin, it does not seem to be associated with either desmoglein I or with isolated desmosomes. Anti-*armadillo* antibodies have been further used to determine the intracellular localization of β-catenin, and to examine its tissue distribution. The implications of these results for the structure and function of different cell–cell adhesive junctions are discussed.

THE evolution of multicellular organisms required that cells acquire the ability to associate and to cooperate in forming different tissues. In both invertebrates and vertebrates, cell association is mediated by cell adhesion molecules of different classes. In epithelial cells, these adhesion molecules comprise well ordered adhesive junctions that have been classified based on their morphological characteristics. For example, many epithelial cells have an adhesive belt encircling each cell that is located near the boundary between their apical and basolateral surfaces, and is known as the adherens junction (or the "zonula adherens," "belt desmosome," or "intermediate junction"). Epithelial cells are also joined by smaller, more tightly organized junctions known as desmosomes (or the "spot desmosomes" or "macula adherens;" reviewed by Schwarz et al., 1990). These two different junctional types each have their own associated protein components.

In the past few years, however, molecular analyses have revealed that some of the component parts of these different types of junctions, though molecularly distinct, are analogous to each other. For example, the transmembrane adhesive components of both adherens-type junctions and desmosomes are members of the cadherin family of cell adhesion molecules (reviewed by Magee and Buxton, 1991). In the case of adherens-type junctions these are the molecules originally defined as cadherins (reviewed by Takeichi, 1991). The desmosomes contain instead the more distantly related desmogleins, desmocollins, and the pemphigus vulgarus antigen (Koch et al., 1990, 1991; Goodwin et al., 1990; Nilles et al., 1991; Wheeler et al., 1991; Collins et al., 1991; Mechanic et al., 1991; Parker et al., 1991; Amagai et al., 1991).

The cytoplasmic domains of different cadherin family members interact with distinct groups of cytoplasmic proteins to form multiprotein complexes (reviewed by Magee and Buxton, 1991). Desmosomes anchor and organize the intermediate filament cytoskeleton, perhaps via the cytoplasmic molecule desmoplakin (Green et al., 1990; Jones and Green, 1991; Stappenbeck and Green, 1992). In contrast, adherens-type junctions link to the actin cytoskeleton, possibly via the vinculin-related molecule α-catenin (Nagafuchi et al., 1991; Herrenknecht et al., 1991).

Immunological criteria identified only one molecule common to both junctional types: plakoglobin (Cowin et al., 1986; Kapprell et al., 1987; Franke et al., 1988). Recently it was found that β-catenin, one of the proteins bound to the cytoplasmic domain of E-cadherin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; Kemler and Ozawa, 1989),

is highly related in protein sequence to plakoglobin (McCrea et al., 1991). Since the sequences of plakoglobin and β-catenin were derived from two different organisms (human plakoglobin [Franke et al., 1989] vs *Xenopus* β-catenin [McCrea et al., 1991]), two different possibilities were raised. One is that plakoglobin and β-catenin are the same protein, with the differences in sequence attributable to divergence between the species. This could provide an explanation for the immunocytochemical demonstration that plakoglobin is present in both adherens-type junctions and desmosomes. The second possibility is that β-catenin and plakoglobin are distinct but related molecules, members of a new multigene family. In this case, the proteins could play similar roles in different junctions.

One difficulty in distinguishing between these possibilities has been the absence of antibodies to β-catenin, a molecule previously defined only by its association with E-cadherin (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; McCrea and Gumbiner, 1991). This also hampered analysis of β-catenin's cell biological properties, tissue distribution, and intracellular localization. This difficulty was circumvented by taking advantage of the fact that a third related molecule exists, the product of the *Drosophila* segment polarity gene *armadillo* (Peifer and Wieschaus, 1990). Antibodies to *Drosophila armadillo* cross react with a vertebrate protein or proteins. In MDCK cells, anti-*armadillo* antibodies distinguish between β-catenin, with which they cross react, and plakoglobin, with which they do not cross react. This reagent was used to demonstrate that plakoglobin and β-catenin represent different members of a multigene family, that β-catenin has properties expected for a junctional protein, and that it is widely distributed in different tissues (presumably interacting with different cadherins). Evidence is presented that other *armadillo* cross reacting molecules are found in some tissues. The implications of these results for the possible function of these molecules in vertebrate and invertebrate adhesive junctions are discussed.

Materials and Methods

Anti-armadillo Antibody

The rabbit polyclonal anti-*armadillo* antibody described by Riggleman et al. (1990), generated using a fusion protein which contains 58 amino acids of the NH_2-terminal domain of *armadillo* fused to β-galactosidase, was used. Most experiments were done with serum that had been absorbed with total *E. coli* protein, and affinity purified by retention on a fusion protein column (Riggleman et al., 1990).

Preparation of Extracts and Immunoblotting

Whole cell extracts were made by rinsing the cells on a 10-cm tissue culture dish, scraping them off with a cell scraper in 1.2× Laemmli buffer, and boiling for 5 min. Mouse tissue extracts were made by dissecting tissue and homogenizing it in an equal volume of 2× Laemmli buffer, followed by boiling. Partially purified desmosomal extracts (kindly provided by R. Foty and M. Steinberg) were prepared according to Blaschuk et al. (1986). SDS-PAGE, Western blotting to nitrocellulose, ^{35}S-Met metabolic labeling, and immunoprecipitations were carried out as previously described (Riggleman et al., 1990; Gumbiner and Simons, 1986). In experiments involving whole cell or tissue extracts, equal amounts of protein were loaded in each lane as assayed by staining the blot with Ponceau S. mAb PG 5.1 (Cowin et al., 1986) was used to immunoblot plakoglobin. Immunoblots were blocked with nonfat dry milk. Anti-*armadillo* primary antibody was used at a dilution of 1:200 to 1:500, while all other primary antibodies used for immunoblotting were used at 1:500, and detected with Promega alkaline phosphatase-conjugated secondary antibodies at 1:7,000 (Promega Corp., Madison, WI).

Cell Fractionation

Separation of cells into soluble and membrane (S100 and P100) fractions was done according to Resh and Erickson (1985). For fractionation with Con A–Sepharose, cells were homogenized with a Dounce homogenizer in radioimmunoprecipitation assay buffer (RIPA) (50 mM Tris, pH 8.5, 300 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS), nuclei were pelleted by centrifugation for 10 min at 10,000 *g*, and then the supernatant was incubated with Con A–Sepharose for 2 h at 4°C. The remaining supernatant was removed and diluted 1:1 with 2× Laemmli buffer, and boiled for 5 min. This served as the unbound fraction. The pellet was then rinsed four times with RIPA buffer, an equal volume of 2× Laemmli buffer was added, and the sample was boiled for 5 min. This was the bound fraction.

Immunoprecipitation with anti-*armadillo* antibody was done in a similar fashion, except the cell homogenate was incubated with anti-*armadillo* antibody (at a 1:10 dilution) for 90 min, and then with Protein A–Sepharose (Sigma Chemical Co., St. Louis, MO) for 1 h. The pellet was washed 4 times with RIPA buffer, an equal volume of 2× Laemmli buffer was added, and the sample was boiled for 5 min. This was the immunoprecipitated fraction.

Xenopus β-catenin was purified as described previously (McCrea and Gumbiner, 1991). For cadherin and desmoglein immunoprecipitations, and for Con-A enrichment of glycoproteins, the mild detergent condition buffer contained 0.5% Triton X-100, 0.01 M Hepes, pH 7.4, 0.15 M NaCl, 2 mM EDTA, 2 mM EGTA, and 0.02% NaAzide and the stringent detergent buffer contained 1% Triton X-100, 0.5% Na deoxycholate, 0.2% SDS, 0.01 M Hepes, pH 7.4, 0.15 M NaCl,, 2 mM EDTA, 2 mM EGTA, and 0.02% NaAzide (McCrea and Gumbiner, 1991). Antibodies for immunoprecipitations were mAb 5D3 for *Xenopus* E-cadherin (Choi and Gumbiner, 1989), mAb rr1 for canine (MDCK) E-cadherin (Gumbiner and Simons, 1986), and rabbit antidesmoglein (Pasdar and Nelson, 1989). For nonimmune control precipitations, an anti–E-cadherin antibody reacting with the incorrect species (*Xenopus* or canine) was used.

Immunofluorescence

MDCK cells and COS-7 cells were both cultured in DME plus 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Double-label indirect immunofluorescence on cells fixed in ice cold methanol was performed as previously described (Angst et al., 1990), with the following exception. Anti-*armadillo* antibody was diluted at 1:10 in TBS, overlaid onto the cells in a moist chamber, and incubated overnight at room temperature. The following day the other primary antibody (anti–E-cadherin or antidesmoplakin) was applied for 1–2 h at 37°C, followed by incubation for 1–2 h in a mixture of the two secondary antibodies. A mouse mAb to desmoplakin (Boehringer Mannheim Biochemicals, Indianapolis, IN) was used at a 1:20 dilution in PBS or TBS. Rat monoclonal E-cadherin antibody (a gift of M. Takeichi) was used at a 1:10 dilution in PBS or TBS. Fluorescein-conjugated anti-rabbit, rhodamine-conjugated anti-mouse (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD), or rhodamine-conjugated anti-rat secondary antibodies (Jackson Laboratories Inc., West Grove, PA) were diluted 1:20 in PBS.

Results

Antibodies to Drosophila armadillo Cross React with a Vertebrate Protein

Polyclonal antibodies directed against 58 amino acids of the NH_2-terminal domain of the *Drosophila* protein *armadillo* have been described previously (Riggleman et al., 1990). These antibodies were affinity purified against the fusion protein antigen and depleted of nonspecific antibodies by preabsorption with *E. coli* proteins. This affinity-purified antiserum specifically recognizes a set of polypeptides in *Drosophila* that have been demonstrated to be the products of the *armadillo* gene (Riggleman et al., 1990). No other *Drosophila* proteins cross react with this antiserum. As *armadillo* protein is highly conserved during the evolution of *Dipteran*

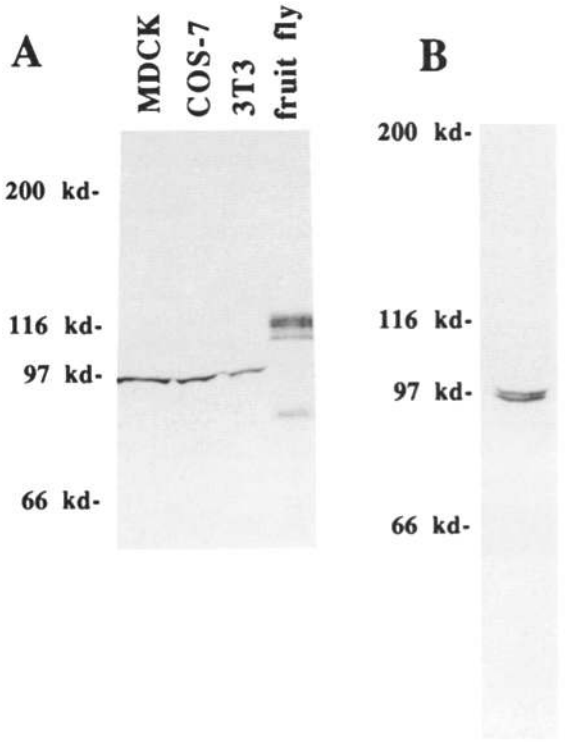

Figure 1. Anti-*armadillo* antibodies cross react with a mammalian protein. (*A*) The cross-reacting protein is found in a number of mammalian cell lines. Approximately equal amounts of whole cell extract from the canine epithelial cell line MDCK, the African Green monkey epithelial cell line COS-7, and the mouse fibroblast cell line 3T3, were separated by SDS-PAGE, alongside a protein extract from *Drosophila* embryos, containing authentic *armadillo* protein. These samples were then immunoblotted with anti-*armadillo* antibody. A cross-reacting protein of a common size is seen in each of the mammalian cell lines. This protein is smaller than the set of posttranslationally modified *armadillo* proteins found in *Drosophila*. (*B*) The cross-reacting mammalian protein can be resolved into two closely spaced bands. MDCK cell extract was separated by SDS-PAGE and immunoblotted with anti-*armadillo* antibody.

insects (M. Peifer and E. Wieschaus, 1992) and has homologs in vertebrates (Peifer and Wieschaus, 1990; McCrea et al., 1991), we tested anti-*armadillo* antiserum to determine whether it cross reacts with any vertebrate proteins.

Mammalian tissue culture cells were tested for cross-reactive proteins. The canine kidney cell line MDCK, the mouse fibroblast line 3T3, and the African green monkey epithelial line COS-7 all have a similar-sized protein with which the antibody strongly reacts (Fig. 1 *A*). No other strongly cross-reacting proteins are apparent in these cell lines. (A similar size cross-reacting protein is also found in the *Xenopus* A6 cell line) (see Fig. 3). Under appropriate gel conditions, the cross-reacting protein can be resolved into a closely spaced pair of bands (Fig. 1 *B*). It seems likely that the reaction of anti-*armadillo* antibody with this protein (or proteins) is significant, as the same or a similar-sized protein reacted with unpurified polyclonal serum from two different mice injected with the same *armadillo* antigen (data not shown).

The Cross-reacting Protein Has Properties Similar to Those of armadillo, β-catenin, and Plakoglobin

Both *armadillo* (Riggleman, 1989; M. Peifer and E. Wieschaus, unpublished data) and plakoglobin (Kapprell et al., 1987) are reversibly bound peripheral membrane proteins, as they are found in both the soluble and membrane-associated fractions. β-catenin and plakoglobin are known to associate with the membrane indirectly via an interaction with transmembrane cell adhesion molecules of the cadherin family (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989; McCrea and Gumbiner, 1991; Korman et al., 1989), E-cadherin and desmoglein-I, respectively. We thus wished to test whether the cross-reacting protein had similar properties. Cells were fractionated into crude membrane and soluble fractions (see Materials and Methods). In MDCK cells, the cross-reacting protein is enriched in the membrane fraction; this enrichment is seen even more dramatically in 3T3 cells (Fig. 2 *A*). This membrane association is also seen in rat liver. In this tissue the cross-reacting protein is highly enriched in the plasma membrane fraction, and absent or nearly absent from the microsomes and nuclear pellet (Fig. 2 *A*).

Two different lines of evidence support the idea that the membrane association of the anti-*armadillo* cross-reacting protein is mediated by a cadherin. First, cells were fractionated into fractions bound and unbound by Con A. Con A is known to bind to cadherins (Peyriéras et al., 1985), and thus precipitates associated proteins (McCrea and Gumbiner, 1991). In both MDCK and 3T3 cells, a fraction of the cross-reacting protein was precipitated by Con A–Sepharose, even under stringent detergent conditions (Fig. 2 *B*). Second, it was demonstrated that a cadherin-like molecule is co-immunoprecipitated with anti-*armadillo* antibody in 3T3 cells (Fig. 2 *C*); this cadherin-like protein was recognized by an antibody directed against a conserved domain of the cadherin molecule, which recognizes many different cadherins (Choi et al., 1990).

armadillo Antibodies Cross React with β-catenin

The relationship in predicted protein sequence between *armadillo*, plakoglobin, and β-catenin (Peifer and Wieschaus, 1990; McCrea et al., 1991) suggested the possibility that the protein recognized by anti-*armadillo* antibody in vertebrate cells might be β-catenin, plakoglobin, or both. We therefore tested whether anti-*armadillo* antibody reacts with β-catenin from the *Xenopus* cell line A6, the source from which β-catenin was originally purified (McCrea and Gumbiner, 1991).

Anti-*armadillo* antibody strongly cross reacts with purified *Xenopus* β-catenin (Fig. 3, lane *1*). β-catenin is likely to be the major cross-reactive protein in these cells, as a protein of the same mobility is recognized by the antibody in the Con A–enriched fraction (Fig. 3, lanes *2* and *3*). Furthermore, a protein of this same size is also recognized by anti-*armadillo* antibody in E-cadherin immunoprecipitates (Fig. 3, lanes *4* and *6*). The cross-reactive protein, like β-catenin, remained bound to E-cadherin even under stringent detergent conditions (Fig. 3, lane *6*). Autoradiography of the immunoprecipitate immunoblotted in Fig. 3, lane *6*, which had been metabolically labeled with [^{35}S]methionine, demonstrated that the only prominent proteins in this sample were E-cadherin and β-catenin (Fig. 3, lane *10*). Fi-

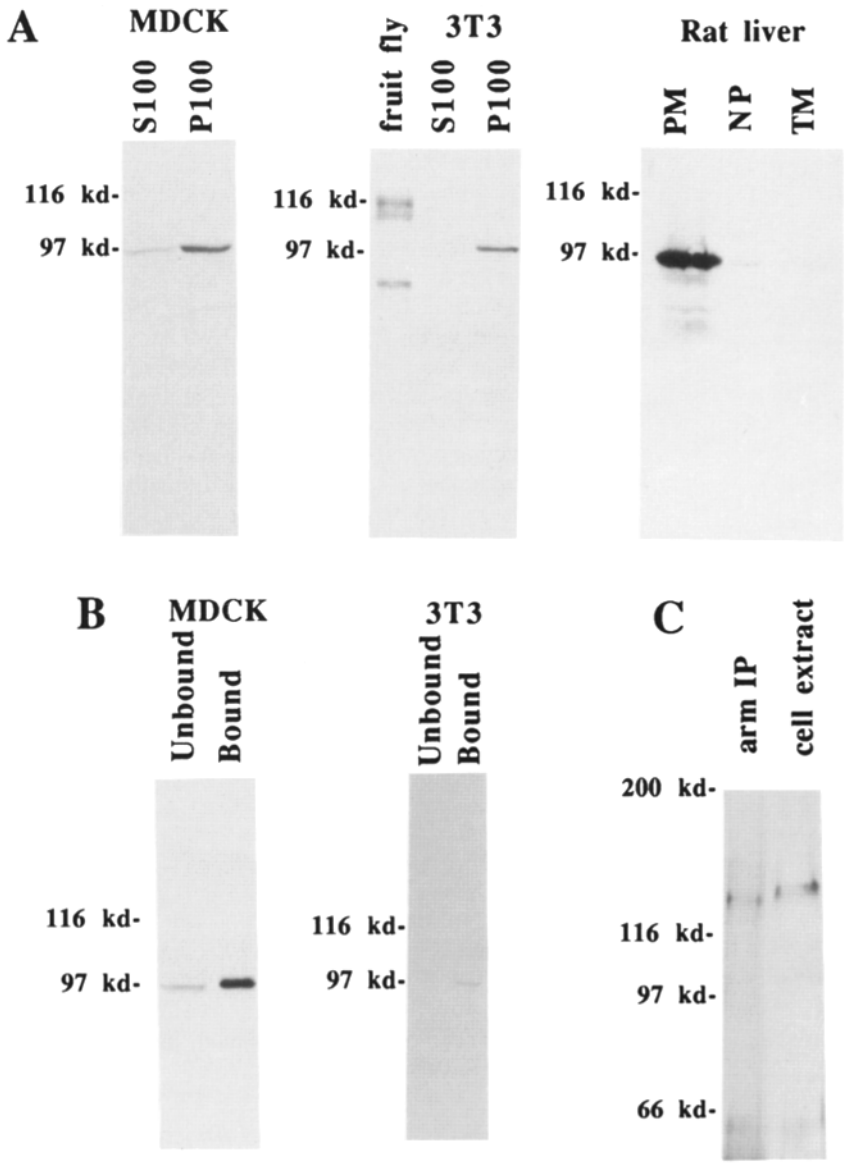

Figure 2. The anti-*armadillo* cross-reacting protein is membrane associated, can be precipitated with Con A–Sepharose, and associates with a cadherin-like molecule. Various fractionated samples were separated by SDS-PAGE and immunoblotted with anti-*armadillo* antibodies. (*A*) Membrane association. MDCK and 3T3 cells were fractionated into crude membrane and soluble fractions (*P100* and *S100*), and approximately equal portions of each fraction separated by SDS-PAGE (the soluble fraction contains substantially more total protein [data not shown]). The cross-reacting protein is enriched in the membrane fraction. Rat liver extracts were separated into plasma membrane, nuclear pellet, and total microsome fractions. The cross-reacting protein is highly enriched in the plasma membrane fraction. (*B*) Con A precipitation. 3T3 or MDCK cell extracts were separated using Con A–Sepharose into bound and unbound fractions. 10% of the unbound fraction and 25% of the bound fraction were separated by SDS-PAGE. A significant fraction of the cross-reacting protein is precipitated with Con A–Sepharose. (*C*) Cadherin association. Proteins were immunoprecipitated from 3T3 cell extracts using anti-*armadillo* antibody, separated by SDS-PAGE, and immunoblotted using an anti-cadherin peptide antibody. Alongside was run whole cell extract from 3T3 cells. The anticadherin peptide antibody detects an ~130 kD protein in both whole cell extracts and anti-*armadillo* immunoprecipitates. Size and cross-reactivity with the anticadherin peptide antibody suggest that this is a cadherin-related molecule. The slight apparent mobility difference between the protein seen in whole cell extracts and that in the immunoprecipitate is probably a gel artifact due to differences in the amount of total protein in the two lanes.

nally, autoradiography of the immunoblot itself (Fig. 3, lane *8*) showed that the anti-*armadillo* cross-reactive protein comigrates with radiolabeled β-catenin. These data together demonstrate definitively that the major cross-reactive protein in *Xenopus* A6 cells is the E-cadherin–associated β-catenin. The anti-*armadillo* antibody also cross reacts with a lower molecular weight polypeptide in the Con A–enriched fraction and in E-cadherin immunoprecipitates, when either was isolated under mild conditions (Fig. 3, lanes *2* and *4*, *asterisk*). However, unlike β-catenin, this polypeptide is not recovered under stringent detergent conditions (Fig. 3, lane *6*). This polypeptide may be a degradation product of β-catenin, but could also be a second cross-reactive molecule. While it is possible that this protein is *Xenopus* plakoglobin, anti-*armadillo* antibody does not cross react with bovine or canine plakoglobin (see below).

β-catenin and Plakoglobin Are Distinct Members of a Multigene Family

The sequence similarity between human plakoglobin and *Xenopus* β-catenin (McCrea et al., 1991) could have two explanations. Plakoglobin and β-catenin could be a single protein identified in different species, or they could be distinct members of a multigene family. Resolution of this issue required a comparison of the two molecules in a single epithelial cell line. As antiplakoglobin antibodies do not cross react with any protein in *Xenopus* A6 cells (McCrea et al., 1991; this may be due to species specificity of the antibodies), plakoglobin and β-catenin were compared in the canine line MDCK, which expresses both desmosomal (Mattey and Garrod, 1986) and cadherin-associated proteins (McCrea and Gumbiner, 1991) (Fig. 4). The anti-*armadillo* cross-reacting protein in MDCK cells (Fig. 4 *a*, lane *3*) comigrates with *Xenopus* β-catenin (Fig. 4 *a*, lane *4*), and also comigrates with the MDCK β-catenin band detected by autoradiography of [^{35}S]methionine-labeled E-cadherin immunoprecipitates from MDCK cells (data not shown). This suggests that in MDCK cells, as in *Xenopus* A6 cells, anti-*armadillo* antibody cross reacts with β-catenin.

In contrast, plakoglobin in MDCK cells, as detected by a

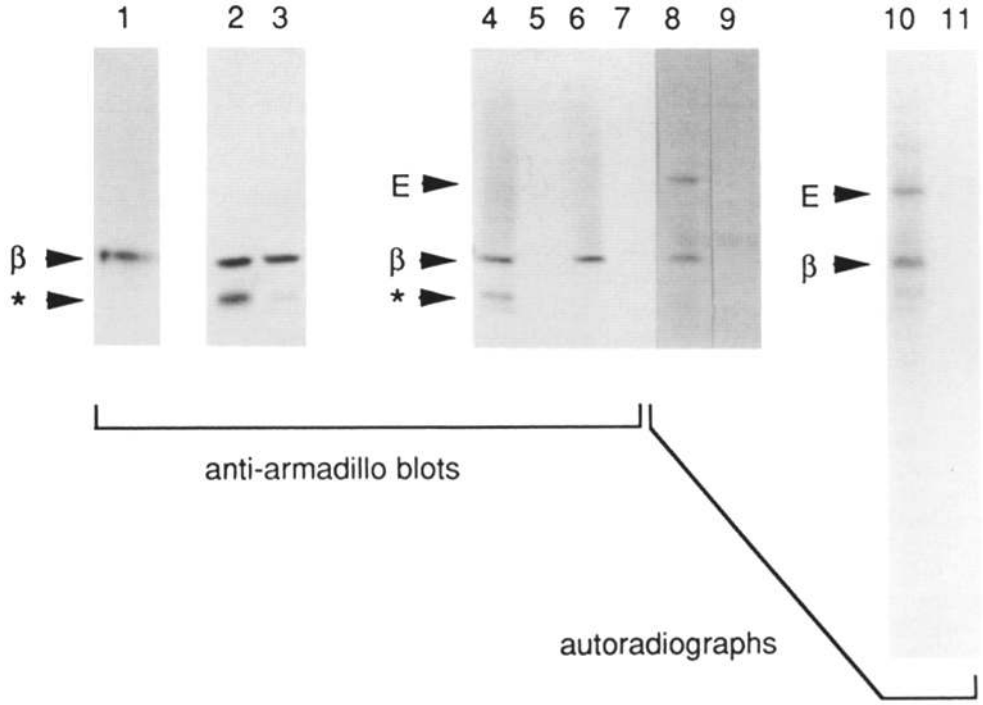

Figure 3. Anti-*armadillo* antibodies recognize *Xenopus* β-catenin. Various samples known to contain β-catenin were separated by SDS-PAGE, and in lanes *1–7* immunoblotted with anti-*armadillo* antibodies. Samples: Lane *1*, ~0.05 μg purified *Xenopus* β-catenin; lane *2*, *Xenopus* A6 cell extract enriched for glycoproteins by binding to Con A–Sepharose and mildly washed; lane *3*, A6 cell extract enriched for glycoproteins by binding to Con A–Sepharose and stringently washed; lane *4*, anti-E–cadherin immunoprecipitate of A6 cell extract, mildly washed; lane *5*, nonimmune antibody control immunoprecipitation for lane *4*; lane *6*, anti-E–cadherin immunoprecipitate of A6 cell extract, stringently washed; lane *7*, nonimmune antibody control immunoprecipitation for lane *6*; lanes *8* and *9*, autoradiography of immunoblot lanes *6* and *7*, respectively, which contained [^{35}S]methionine-labeled samples; lanes *10* and *11*, the samples in lanes *6* and *7* were run on a separate gel and autoradiographed directly without immunoblotting. (*β*) β-catenin, ~92 kD; (*E*) E-cadherin, ~140 kD; (*) secondary immunoreactive band (see text).

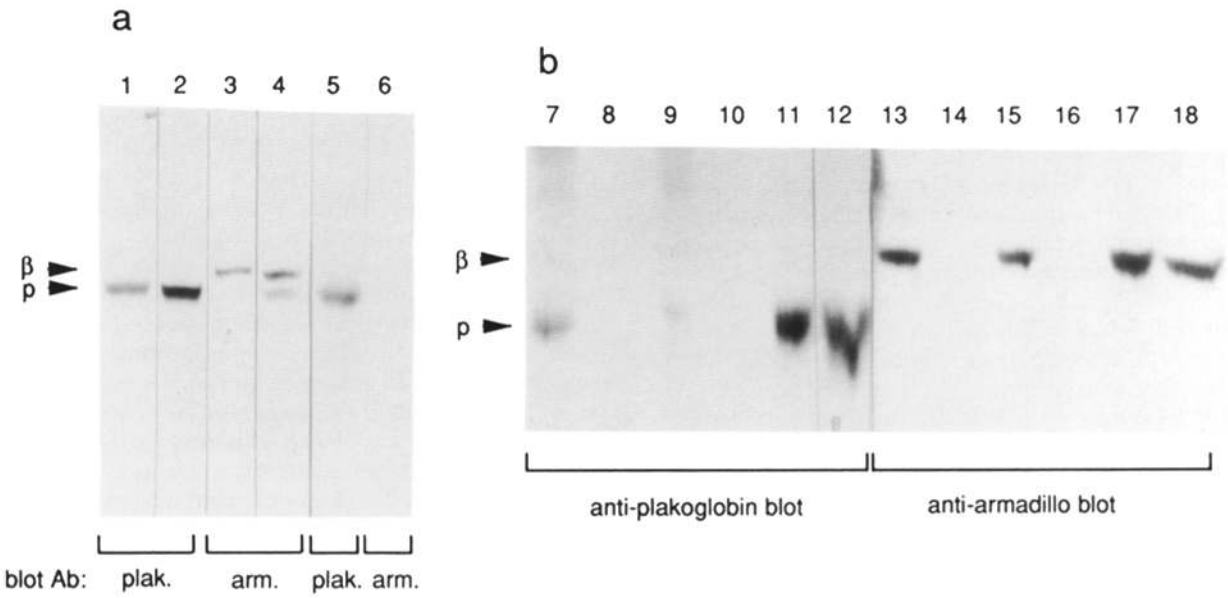

Figure 4. In canine MDCK cells, anti-*armadillo* and antiplakoglobin antibodies recognize distinct proteins associated with E-cadherin. (*a*) Comparison of proteins recognized by anti-*armadillo* and antiplakoglobin antibodies. Various samples were immunoblotted with either anti-*armadillo* polyclonal antibody (lanes *3*, *4*, and *6*) or with antiplakoglobin mAb (lanes *1*, *2*, and *5*). Samples: lane *1*, MDCK whole cell extract; lanes *2* and *3*, Con A–Sepharose enriched glycoproteins from MDCK cell extract; lane *4*, Con A–Sepharose enriched glycoproteins from *Xenopus* A6 cell extract; lanes *5* and *6*, partially purified bovine desmosome fraction. (*b*) Co-immunoprecipitation of β-catenin and plakoglobin with E-cadherin from MDCK cells. Samples were immunoblotted with either antiplakoglobin mAb (lanes *7–12*) or with anti-*armadillo* polyclonal antibody (lanes *13–18*). Samples: lanes *7* and *13*, anti-E–cadherin immunoprecipitates, mildly washed; lanes *8* and *14*, nonimmune antibody control immunoprecipitations for lanes *7* and *13*; lanes *9* and *15*, anti-E–cadherin immunoprecipitates, stringently washed; lanes *10* and *16*, nonimmune antibody control immunoprecipitations for lanes *9* and *15*; lanes *11* and *17*, Con A–Sepharose enriched glycoproteins; lanes *12* and *18*, whole MDCK cell extract. (*β*) β-catenin; (*p*) plakoglobin.

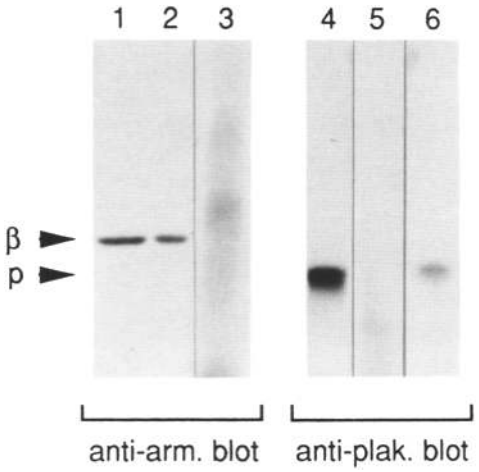

Figure 5. Plakoglobin, but not β-catenin, immunoprecipitates with desmoglein I. MDCK cell extracts were immunoprecipitated with either anti-E-cadherin (lanes *2* and *5*) or antidesmoglein I (lanes *3* and *6*), or were precipitated with Con A–Sepharose (lanes *1* and *4*). The samples were then immunoblotted with either anti-*armadillo* (lanes *1–3*) or antiplakoglobin (lanes *4–6*). (*β*) β-catenin; (*p*) plakoglobin. Samples containing desmoglein I immunoprecipitates were derived from four times as much cell extract than the other lanes, because of the poor solubility of desmosomes.

plakoglobin mAb, migrates distinctly faster that β-catenin, with a molecular mass of ~85 kD (Fig. 4, lanes *1* and *2*; contrast also the mobility of plakoglobin in Fig. 4 *b*, lanes *7*, *11*, and *12*, with that of β-catenin in lanes *13*, *15*, *17*, and *18*). Furthermore, anti-*armadillo* antibody fails to recognize plakoglobin in a purified bovine desmosome fraction (Fig. 4 *a*, lane *6*), even though it was readily detected by the plakoglobin antibody (Fig. 4 *a*, lane *5*). Thus anti-*armadillo* and antiplakoglobin antibodies allow us to distinguish β-catenin and plakoglobin in a single epithelial cell line.

Two of us had previously reported that plakoglobin co-immunoprecipitates from MDCK cells with E-cadherin (McCrea et al., 1991). Given the ability to distinguish β-catenin from plakoglobin, this issue was reexamined, probing E-cadherin immunoprecipitates for the presence of β-catenin (using anti-*armadillo* antibody) or plakoglobin (using plakoglobin antibody) (Fig. 4 *b*). β-catenin co-immunoprecipitates specifically with E-cadherin under mild detergent conditions (Fig. 4 *b*, lane *13*), and, as expected, remains associated with E-cadherin under more stringent detergent conditions (Fig. 4 *b*, lane *15*). Plakoglobin is also detectable in E-cadherin immunoprecipitates obtained under mild detergent conditions (Fig. 4 *b*, lane 7). This coprecipitation is specific, as it is not detected when a nonimmune control is used (Fig. 4 *b*, lane *8*). In contrast to β-catenin, however, only a small amount of plakoglobin remains associated with E-cadherin under stringent detergent conditions (Fig. 4 *b*, lane *9*). In several different experiments, the recovery of plakoglobin in E-cadherin immunoprecipitates under stringent conditions was variable, and sometimes not detectable (e.g., Fig. 5, lane *5*). Thus, although β-catenin is the major protein associated with E-cadherin under stringent conditions, plakoglobin also seems to be present in the E-cadherin–catenin protein complex as a more minor and/or loosely associated complex. This association is consistent with the possibility that plakoglobin is the same as γ-catenin, a component of the E-cadherin–catenin protein complex whose molecular nature remains to be determined (Ozawa et al., 1989).

Plakoglobin is found localized at both the desmosome and the adherens-type junctions in epithelial cells (Cowin et al., 1986), and it co-immunoprecipitates with both desmoglein I (Korman et al., 1989) and E-cadherin (these data). It was of interest, therefore, to determine whether β-catenin can also associate with desmosomal proteins. As was mentioned above, β-catenin is not detected in significant quantities in isolated bovine desmosomes (Fig. 4 *a*, lane *6*). Consistent with this, β-catenin is also not detected in desmoglein I immunoprecipitates of MDCK cells under stringent conditions (Fig. 5, lane *3*); plakoglobin is detected, as expected in these desmoglein immunoprecipitates (Fig. 5, lane *6*). Thus, β-catenin does not seem to be a significant component of desmosomes or tightly associated with the desmosomal glycoprotein desmoglein I. However, because of the poor solubility of the desmosomes, it is possible that the extraction conditions used for immunoprecipitations may only solubilize a specific subfraction of desmosomes or perhaps only newly synthesized desmosomal components, which might not contain β-catenin. However, the absence of anti-*armadillo* cross-reacting material in isolated bovine desmosomes supports the idea that it does not interact significantly with any desmosomal protein.

β-catenin Localizes to Cell–Cell Junctions

To further investigate the cellular localization of β-catenin, single and double-label immunofluorescence analyses of MDCK and COS-7 epithelial cells (derived from canine and monkey kidney, respectively) were carried out. In both cases β-catenin protein, as detected with anti-*armadillo* antibody, localized to the cell surface at sites of cell–cell contact (Fig. 6, *a*, *c*, and *e*). When the anti-*armadillo* antibody was used in conjunction with an antibody directed against E-cadherin, the distribution of β-catenin appeared to coincide with E-cadherin at cell interfaces (Fig. 6, *a* and *b*). These observations are consistent with β-catenin being localized to adherens-type junctions, although labeling of other regions of the lateral plasma membrane cannot be ruled out.

Double labels were also performed using antibodies against desmoplakin, a desmosomal plaque protein. In COS-7 cells, anti-*armadillo* antibody staining involved considerably more cell surface area than desmoplakin staining (Fig. 6, *e* and *f*), in line with previous observations that desmosomes are infrequent in COS-7 cells growing at low density (Stappenbeck and Green, 1992). This result suggests that β-catenin is present in nondesmosomal junctions. In both COS-7 and MDCK cells, along cell interfaces where both anti-*armadillo* and antidesmoplakin staining were evident, antidesmoplakin antibody staining could be seen in some cases to fill the spaces where anti-*armadillo* staining was diminished or absent, and vice versa (Fig. 6, *c–f*). This observation suggests that β-catenin is not concentrated in desmosomes.

β-catenin Is Found in a Wide Variety of Tissue Types

The known members of the cadherin family have distinct, if overlapping, tissue distributions. To determine the tissue distribution of β-catenin, a variety of mouse tissues were compared, using the anti-*armadillo* antibody (Fig. 7). Most tis-

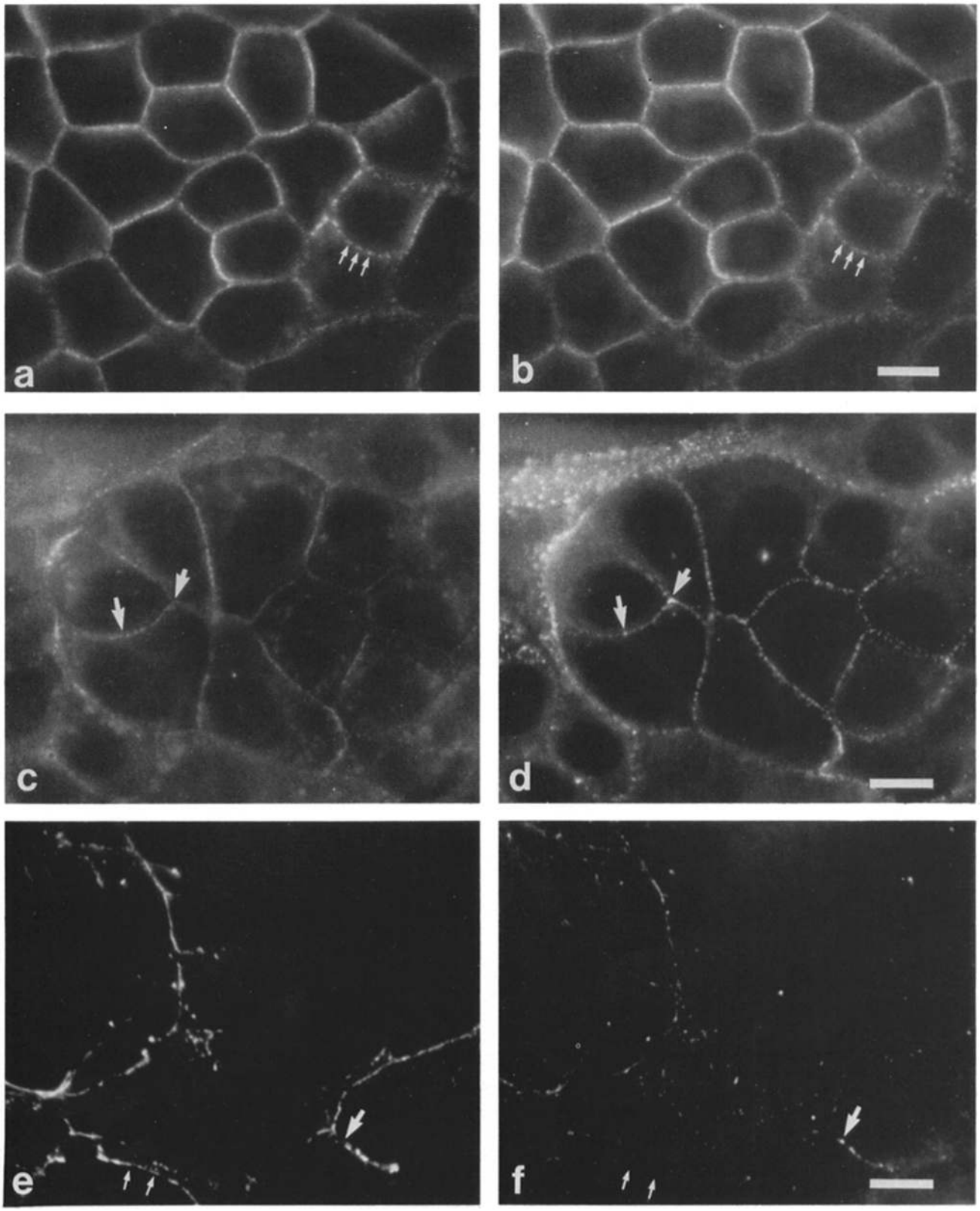

Figure 6. Cellular localization of β-catenin. Double-label immunofluorescence analysis of MDCK (*a–d*) and COS-7 (*e* and *f*) cells. Cells in *a*, *c*, and *e* were stained with anti-*armadillo* antibody. Cells in *b* were stained with anti-E-cadherin antibody, and cells in *d* and *f* with antidesmoplakin antibody. Note coincidence of staining pattern in *a* and *b* (*small arrows*). Staining with both anti-E-cadherin and with anti-*armadillo* in MDCK cells was often relatively uniform along the cell surface. We have chosen a region where staining is more nonuniform, to illustrate the colocalization. Large arrows in *c–f* denote regions of intense reaction with desmoplakin antibody and reduced staining with anti-*armadillo* antibody. In COS-7 cells, many cell borders are devoid of desmoplakin staining, although β-catenin is present (*small arrows*). Bars, 10 μm.

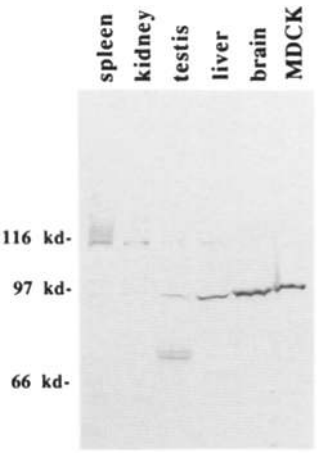

Figure 7. Tissue distribution of β-catenin. Protein extracts were made by Dounce-homogenizing dissected murine tissues directly in SDS-gel sample buffer. Approximately equal amounts of protein derived from the indicated murine tissues or from MDCK cells were separated by SDS-PAGE and immunoblotted with anti-*armadillo* antibody. While a protein comigrating with β-catenin from MDCK cells is present in all tissues, it differs in abundance, and in some tissues (e.g., *spleen*) it does not represent the major cross-reactive species. The larger cross-reacting proteins found in tissues such as spleen may represent other members of the *armadillo*/β-catenin/plakoglobin family, or may be fortuitously cross-reacting molecules.

sues examined express a protein of identical size to that found in cultured cells, presumably representing β-catenin. Some tissues, such as the brain, are rich sources of this protein. Other tissues, like the liver and testes, have lower amounts of the same or similar-sized protein. Intriguingly, a few tissues show cross-reacting proteins of other sizes. This is most apparent in spleen cells, in which a set of prominent proteins are seen, ranging from 115–120 kD. It will be interesting to determine whether these represent related proteins or nonspecific cross-reactivity. In some tissues, notably testes, proteins smaller than β-catenin cross react with the antibody; these may represent proteolytic breakdown products.

Discussion

Antibody Directed Against the Drosophila Segment Polarity Gene armadillo Cross Reacts with β-catenin

The *Drosophila* segment polarity gene *armadillo* is required for patterning within embryonic and adult segments, and is necessary for the proper reception/interpretation of the intercellular signal encoded by *wingless*, homolog of vertebrate *wnt-1* (Wieschaus et al., 1984; Wieschaus and Riggleman, 1987; Peifer et al., 1991). *armadillo's* close relationship to the vertebrate adhesive junction proteins plakoglobin and β-catenin (Peifer and Wieschaus, 1990; McCrea et al., 1991) led us to examine whether *armadillo* antibody cross reacts with any vertebrate protein(s).

Anti-*armadillo* antibody cross reacts with a protein found in a variety of vertebrate cell lines (Figs. 1 and 3), which differ in tissue (fibroblasts, kidney, and epidermal cells) and species of origin (mouse, monkey, dog, and frog). The apparent molecular mass of this protein is distinct from that of plakoglobin (81–83 kD; Gorbsky et al., 1985; Cowin et al., 1986). The size of the cross-reacting protein is more consistent with the idea that it is β-catenin (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989; McCrea and Gumbiner, 1991).

We confirmed that the cross-reacting protein is β-catenin by showing that anti-*armadillo* antibody cross reacts with purified β-catenin from *Xenopus* cells, and with β-catenin co-immunoprecipitated with anti-E cadherin antibody from both *Xenopus* and MDCK cells (Figs. 3 and 4). Size and co-immunoprecipitation with E-cadherin under stringent conditions are the defining features of β-catenin. In retrospect, this cross-reaction is not too surprising, as the portion of *armadillo* against which the antibody was raised is well conserved between *Drosophila armadillo* and *Xenopus* β-catenin (59% identical; and not nearly as well conserved in plakoglobin, 33% identical, Fig. 8). We will consider for the rest of this discussion that the major cross-reacting protein is β-catenin.

β-catenin and Plakoglobin Are Distinct Members of a Multigene Family

The sequence similarity between the *Xenopus* adherens junction protein β-catenin (McCrea et al., 1991) and the human desmosomal protein plakoglobin (Franke et al., 1989) had two possible explanations. One was that β-catenin and plakoglobin were the same molecule, and that differences in sequence reflected divergence between frogs and humans. This would have been consistent with the fact that plakoglobin is the only desmosomal component found in adherens-type junctions (Cowin et al., 1986; Kapprell et al., 1987; Franke et al., 1988). The second possibility was that β-catenin and plakoglobin are related but distinct members of a multigene family.

While antibody to plakoglobin is available (Cowin et al., 1986), lack of antibody to β-catenin hampered resolution of this point. This difficulty was circumvented by the use of antibody directed against *armadillo* that cross reacts with purified *Xenopus* β-catenin. In contrast, anti-*armadillo* antibody does not cross react with partially purified bovine desmosomal extracts or with antidesmoglein immunoprecipitates of MDCK cells, both of which contain plakoglobin (Figs. 4 and 5) Using both anti-*armadillo* and antiplakoglobin antibodies, it was shown that a single cell type, MDCK cells, can contain both plakoglobin and β-catenin, which are quite distinct in size (Fig. 4). This clearly demonstrates that in canine cells, β-catenin and plakoglobin represent different, though related proteins. It is possible that the lower molecular weight molecule that weakly cross reacts with anti-*armadillo* antibody in *Xenopus* A-6 cells (e.g., Fig. 3, lanes 2 and 4, *asterisk*) is *Xenopus* plakoglobin, but this seems unlikely, due to the lack of cross-reactivity with plakoglobin in MDCK cells or bovine desmosomes.

Using anti-*armadillo* antibody, some biochemical properties of β-catenin were documented. These properties are consistent with β-catenin playing a role in adherens-type junctions similar to that played by plakoglobin in desmosomes. Like plakoglobin (Kapprell et al., 1987), a large fraction of β-catenin fractionates with membranes (Fig. 2 *A*). By using immunofluorescence, it was demonstrated that β-catenin is found at the plasma membrane in regions of cell–cell contacts. It is enriched in structures which appear to be adherens-type junctions, by virtue of colocalization with E-cadherin (Fig. 6). β-catenin is localized in areas of the membrane which often seem to be distinct at the light microscope level from those occupied by the desmosomal plaque protein desmoplakin (Fig. 6). Thus plakoglobin and β-catenin are both assembled into adhesive junctions via an inter-

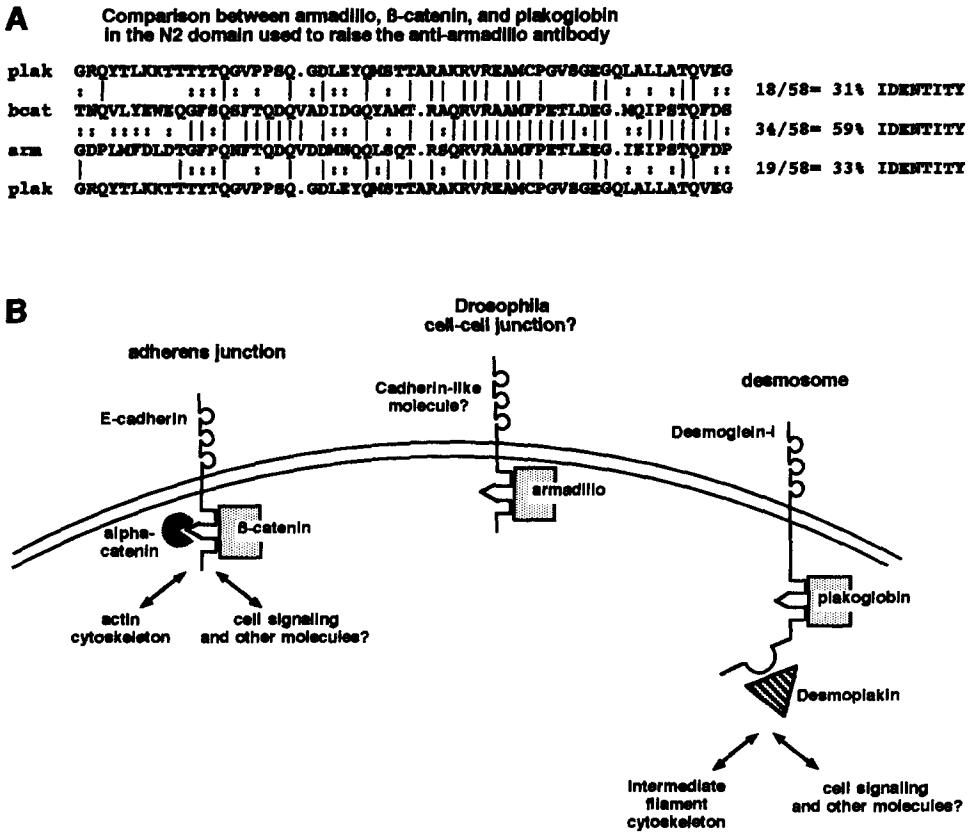

Figure 8. Comparison among members of the *armadillo*/β-catenin/plakoglobin family. (*A*) Sequence comparison of *armadillo*, β-catenin, and plakoglobin in the region against which the anti-*armadillo* antibody is directed. Note that *armadillo* and β-catenin are much more closely related in this region than are *armadillo* and plakoglobin. (*B*) Possible parallels in the structure of different cell–cell adhesive junctions. Adherens junctions and desmosomes are shown, along with a hypothetical *Drosophila* cell–cell adhesive junction. Adherens junctions and desmosomes share transmembrane adhesive molecules of the cadherin family, "true" cadherins and the cadherin-related desmogleins, respectively. Their related extracellular domains are thought to interact with cadherins or desmogleins on other cells, and thus to mediate adhesion. The intracellular domains of the cadherin family members are thought to mediate assembly of multiprotein complexes. The related molecules β-catenin and plakoglobin each interact with the cytoplasmic domains of these cadherin family members, presumably via conserved regions. Other proteins such as the adherens-junction protein a-catenin also interact in this region. Desmoglein I has a COOH-terminal extension which may be the site of interaction of the desmosomal molecule desmoplakin. These cytoplasmic protein complexes are thought to mediate cytoskeletal association (with the actin cytoskeleton in the adherens junction and the intermediate filament cytoskeleton in the desmosome), and may also mediate other functions. We believe it is possible that *armadillo* may interact with a cadherin-like molecule in a hypothetical *Drosophila* cell–cell adhesive junction.

action with a transmembrane glycoprotein of the cadherin family.

Is β-catenin the True Homolog of armadillo?

The ability of anti-*armadillo* antibody to cross react with β-catenin and not plakoglobin raises the question of whether β-catenin is the true *armadillo* homolog. As mentioned above, *armadillo* is considerably more similar to β-catenin than to plakoglobin in the region of the NH_2-terminal domain to which the antibody was raised (Fig. 8). This greater similarity between *armadillo* and β-catenin extends through the entire NH_2-terminal domain, and contributes to the fact that there is significantly greater similarity throughout the protein sequence between *armadillo* and β-catenin (71% identity) than between *armadillo* and plakoglobin (63% identity). However, the identity between *armadillo* and β-catenin (71% identity) is only slightly greater than that between β-catenin and plakoglobin (68% identity). This ambiguity in sequence comparison is compounded by the existence of other proteins that cross react with *armadillo* antibody in some mammalian tissues (some as large as *Drosophila armadillo*, e.g., *spleen* in Fig. 7), raising the possibility that there exist other members of the β-catenin/plakoglobin multigene family, some of which may be even more closely related in sequence and function to *armadillo*. The answer to the question of which of these proteins, if any, represents the "true" *armadillo* homolog will ultimately rest on a more careful analysis of the roles of *armadillo* and its vertebrate homologs in insect and vertebrate cells.

Different Adhesive Junctions Share Distinct but Analogous Components

The past two years reshaped the view of adhesive junctions. Different adhesive junction types had been viewed as separate entities, with distinct structures and functions. However, molecular analyses revealed that these junctions, while morphologically distinct, contain analogous and often related proteins. Both junctional types are organized around transmembrane adhesive molecules of the cadherin family, "classical" cadherins in adherens-type junctions, and the cadherin-related desmoglein/desmocollins in desmosomes (Fig. 8; reviewed by Magee and Buxton, 1991). Other junctional proteins are analogous in function, while differing in sequence. In adherens junctions, the vinculin-related α-catenin may link cadherin to the actin cytoskeleton (Nagafuchi et al., 1991), while the linkage between desmosomes and intermediate filaments may be mediated by desmoplakin (Green et al., 1990; Jones and Green, 1991; Stappenbeck and Green, 1992).

Plakoglobin and β-catenin are among the other adhesive

junction components. Each physically interacts with cadherin family members, β-catenin with "classic" cadherins (Ozawa et al., 1989; Nagafuchi and Takeichi, 1989), and plakoglobin with desmoglein I (Korman et al., 1989). Deletion of E-cadherin's catenin-binding domain abrogates cell–cell adhesion (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989), suggesting that cadherin–catenin association is key to assembling an effective junction. However, the precise roles of β-catenin and plakoglobin have yet to be elucidated. One suggestion is that they help assemble individual cadherin molecules into the complex multiprotein structures that comprise adhesive junctions, by facilitating cadherin–cadherin interactions or by serving as a bridge between cadherins and other junctional proteins.

The widespread tissue distribution of β-catenin (Fig. 7) suggests that it is present in cells expressing a wide variety of cadherins. The tissue richest in β-catenin is brain, where numerous cadherins are expressed (Hatta et al., 1988; Suzuki et al., 1991). Others have found that similar size proteins co-immunoprecipitate with E- and N-cadherins (Wheelock and Knudsen, 1991; Ozawa and Kemler, 1992). It thus seems likely that many, if not all, cadherins will associate with similar catenin molecules, via their highly conserved cytoplasmic domain.

In a simple world, β-catenin and plakoglobin would be junction specific, β-catenin associated with cadherins in adherens junctions, and plakoglobin associated with desmoglein I in desmosomes. This simple picture is incorrect. While β-catenin cannot be detected in desmosomal extracts (Fig. 4 *a*, lane *6*), anti-E-cadherin immunoprecipitates contain variable amounts of plakoglobin, in addition to β-catenin (although the plakoglobin association appears weaker). Plakoglobin can thus associate with different cadherin family members, consistent with its presence in both desmosomes and adherens-type junctions (Cowin et al., 1986; Franke et al., 1988). Plakoglobin may even be the previously identified E-cadherin associated molecule γ-catenin. It remains possible, however, that co-immunoprecipitation under nonstringent conditions of plakoglobin with E-cadherin could result from a more indirect interaction. It is worth noting that a glycoprotein immunologically related to E-cadherin was reported to be present in desmosomes (Jones, 1988). These results also do not rule out the possibility that β-catenin interacts with the desmocollins, which also contain the conserved cadherin cytoplasmic domain. However, the failure to detect β-catenin in crude desmosomal extracts makes this less likely.

The relationship among *armadillo*, β-catenin, and plakoglobin provided a link between *wingless/wnt-1*–mediated cell–cell signaling and cell adhesion. This relationship, and the discovery that *wnt-1* is involved in patterning during vertebrate development (McMahon and Moon, 1989; McMahon and Bradley, 1990; Thomas and Capecchi, 1990), provide further evidence that vertebrate and invertebrate cells share underlying cell–cell communication machinery that is used to regulate pattern formation during early development.

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