

Interaction of α -Actinin with the Cadherin/Catenin Cell–Cell Adhesion Complex via α -Catenin

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Abstract. Cadherins are Ca^{2+} -dependent, cell surface glycoproteins involved in cell–cell adhesion. Extracellularly, transmembrane cadherins such as E-, P-, and N-cadherin self-associate, while intracellularly they interact indirectly with the actin-based cytoskeleton. Several intracellular proteins termed catenins, including α -catenin, β -catenin, and plakoglobin, are tightly associated with these cadherins and serve to link them to the cytoskeleton. Here, we present evidence that in fibroblasts α -actinin, but not vinculin, colocalizes extensively with the N-cadherin/catenin complex. This is in contrast

to epithelial cells where both cytoskeletal proteins colocalize extensively with E-cadherin and catenins. We further show that α -actinin, but not vinculin, co-immunoprecipitates specifically with α - and β -catenin from N- and E-cadherin-expressing cells, but only if α -catenin is present. Moreover, we show that α -actinin coimmunoprecipitates with the N-cadherin/catenin complex in an actin-independent manner. We therefore propose that cadherin/catenin complexes are linked to the actin cytoskeleton via a direct association between α -actinin and α -catenin.

THE cadherins form a family of cell surface glycoproteins that function in promoting Ca-dependent cell–cell adhesion and serve as the transmembrane components of cell–cell adherens junctions (Takeichi, 1988; Geiger, 1989; Geiger et al., 1990; Takeichi, 1991; Geiger and Ayalon, 1992; Grunwald, 1993). Adherens junctions are found between many cell types, including epithelial cells, cardiac myocytes, and fibroblasts (Volk and Geiger, 1984; Geiger et al., 1987, 1990; Geiger and Ayalon, 1992; Heaysman and Pegrum, 1973). Extracellularly the individual cadherins self-associate to promote specific cell–cell interactions (Nose et al., 1988, 1990; Friedlander et al., 1989), while intracellularly they interact with a group of proteins, collectively termed catenins (Ozawa and Kemler, 1992; Ozawa et al., 1989; Magee and Buxton, 1991). The catenins (α , β , and γ) are thought to link cadherins to the actin-based cytoskeleton, although the mechanism is not understood. Both the cadherin cytoplasmic domain and the associated catenins have been shown to be required for full cadherin activity (Nagafuchi and Takeichi, 1988, 1989; Ozawa et al., 1989, 1990; Jaffe et al., 1990; Tsukita et al., 1992; Hirano et al., 1992).

α -Catenin is a 102-kD protein with homology to vinculin (Nagafuchi et al., 1991; Herrenknecht et al., 1991). β -cate-

nin is a 92-kD protein related to both the protein product of *armadillo*, a *Drosophila* segment polarity gene, and plakoglobin (Peifer and Wieschaus, 1990; McCrea et al., 1991), a vertebrate protein found at desmosomes and cell–cell adherens junctions (Cowin et al., 1986). Plakoglobin also associates with cadherins and is thought to be the same as γ -catenin (Peifer et al., 1992; Knudsen and Wheelock, 1992).

A variety of cell types express cadherins that have been shown to promote specific cell–cell adhesion. E-cadherin is the major cadherin expressed by polarized epithelial cells, whereas both E- and P-cadherin are expressed by squamous epithelial cells (reviewed by Takeichi, 1988). N-cadherin is expressed by developing and mature cardiomyocytes (Volk and Geiger, 1984) and has been shown to play an important role in promoting both myocyte interaction and myofibrillogenesis (Peralta Soler and Knudsen, 1994). N-cadherin, along with other adhesion molecules, functions as a cell–cell adhesion molecule in nerve (Matsunaga et al., 1988) and in developing skeletal muscle (Knudsen et al., 1990). N-cadherin has been shown to be expressed by primary chicken fibroblasts and P-cadherin by a normal rat fibroblast cell line (Hirano et al., 1987; Geiger et al., 1990; Itoh et al., 1991).

Cadherins localize to the cell–cell adherens junctions in epithelial cells and cardiomyocytes. At the electron microscopic level, these junctions are characterized by close plasma membrane apposition between interacting cells and by the presence of electron dense material at the intracellular face of the plasma membrane. The dense, submembranous material, sometimes referred to as the plas-

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malemmal undercoat, contains a number of cytoplasmic proteins, many of which are well characterized (Tsukita et al., 1989, 1992; Tsukita et al., 1993). Examples include α -catenin, β -catenin, plakoglobin, vinculin, α -actinin, radixin, the 220-kD protein (ZO-1), zyxin, spectrin, and actin. Exactly how these various proteins interact with one another is an area of on-going investigation.

Some interactions among these cytoplasmic proteins have been described, whereas others are still a matter of speculation. For example, vinculin and zyxin have both been shown to bind to α -actinin (Belkin and Koteliensky, 1987; Wachsstock et al., 1987; Crawford et al., 1992; McGregor et al., 1994). Vinculin (Menkel et al., 1994) and α -actinin (Burridge and Feramisco, 1982; Blanchard et al., 1989) both interact with actin. Radixin caps the barbed end of actin filaments (Tsukita et al., 1989) and the 220-kD protein may interact with spectrin (Itoh et al., 1991). Due to homology between α -catenin and a domain in vinculin thought to be involved in vinculin self-association, α -catenin has been proposed to self-associate and thereby consolidate cadherins in the adherens junction (Nagafuchi et al., 1991; Herrenknecht et al., 1991). Alternatively, α -catenin has been proposed to bind to vinculin and thereby link the cadherin/catenin complex to the cytoskeleton. However, no evidence has been presented to date to support either of these possibilities.

In this paper we show that α -actinin, but not vinculin, colocalizes extensively with the N-cadherin/catenin complex expressed by fibroblasts. This is in contrast to epithelial cells where both α -actinin and vinculin colocalize with the E-cadherin/catenin complex. We present evidence that α -actinin, but not vinculin, coimmunoprecipitates specifically with α - and β -catenin from N- and E-cadherin-expressing cells, but only if α -catenin is present. In addition, we present evidence that α -actinin associates with the N-cadherin/catenin complex in a manner not dependent upon the presence of actin. We therefore propose that cadherin/catenin complexes are linked to the actin cytoskeleton via a direct interaction between α -catenin and α -actinin.

Materials and Methods

Cells

The WI-38 normal human embryonic lung fibroblast cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD) and was maintained in minimum essential medium Eagle (Autopow; Flow Labs., Inc., McLean, VA) with 2 \times essential and nonessential amino acids, and 2 \times vitamins, 20% FBS. The HeLa human carcinoma cell line (CCL 2) was obtained from ATCC and was grown in Dulbecco's modified Eagle medium (Sigma Immunochemicals, St. Louis, MO) containing 10% FBS. The E-cadherin-expressing human choriocarcinoma epithelial cell line, JAR PR497 (JAR), was cultured as described (Wheelock et al., 1987). The E-cadherin-expressing PC9 lung carcinoma cell line, which does not express α -catenin (Shimoyama et al., 1992), was a gift of Dr. Eric Holmes (University of Washington, Seattle, WA) and was grown in the same medium used for the WI-38 cells. Cells were metabolically radiolabeled with [³⁵S]-methionine/cysteine as previously described (Wheelock, 1990).

Antibodies and Other Reagents

The rat monoclonal anti-human α -actinin (E2) has been described (Wheelock, 1990). Mouse monoclonal anti- α -actinin (BM-75.2), anti-(chicken)vinculin (VIN-11-5), anti-(human) vinculin (hVIN-1), and anti- β -actin (AC-15) were purchased from Sigma. The BM-75.2 anti- α -actinin is a high titer IgM isotype antibody. Mouse mAbs to α -catenin (1G5) and β -catenin

(9F2, 12F7, and 10C4) have been described (Johnson et al., 1993). The 9F2 and 12F7 mAbs are specific for β -catenin, whereas 10C4 recognizes both β -catenin and plakoglobin. Rabbit polyclonal antibodies to α - and β -catenin were gifts from Drs. B. Gumbiner (Sloan-Kettering Institute, NY) and P. McCrea (University of Texas, Houston, TX). The mouse monoclonal anti-plakoglobin, PG5.1, was a gift from Dr. W. Franke (German Cancer Research Center, Heidelberg). Mouse mAb 15F11 to plakoglobin was generated as described (Johnson et al., 1993). Polyclonal anti-pan-cadherin, which recognizes N-, E-, and P-cadherin, was generated by injecting a rabbit with the cytoplasmic domain of human N-cadherin generated and purified using the pMAL expression system (New England Biolabs, Beverly, MA). Rabbit polyclonal anti-E-cadherin has been described (Wheelock et al., 1987). The monoclonal antibody recognizing N-cadherin (13A9), but not P-, E-, or M-cadherin, was generated by injecting mice with a cytoplasmic fragment of human N-cadherin expressed and purified using the pMAL expression system. The cDNA encoding human N-cadherin (Reid and Hemperly, 1990) was a gift from Dr. J. Hemperly (Becton Dickinson and Company Research Center, Research Triangle Park, NC).

Extraction of Cells

Cells were extracted in several different ways, as indicated throughout the text and figure legends. For some experiments, monolayers of cells were washed three times with PBS at room temperature and were extracted on ice with 2 ml/75-cm² flask 10 mM Tris acetate, pH 8.0, containing 0.5% NP-40 (BDH Chemicals Ltd., Poole, England), 1 mM EDTA, and 2 mM PMSF. The cells detached from the tissue culture flask after a few minutes in this buffer and were agitated by vigorous pipetting for 10 min on ice. Insoluble material was removed by centrifugation at 15,000 g for 45 min. Alternatively, confluent cultures of cells were washed three times with PBS, scraped gently into PBS on ice, pelleted by brief centrifugation, and extracted with agitating for 15 min on ice in a fivefold excess (vol/vol) of 10 mM Tris acetate, pH 8.0, containing 0.5% NP-40, 0.1 mM Ca²⁺, 20 μ g/ml leupeptin, and 2 mM PMSF. Insoluble material was removed by centrifugation at 15,000 g for 30 min. For other experiments, cells were washed with PBS and scraped into 10 mM Tris-HCl, pH 7.5, containing the protease inhibitors PMSF, leupeptin, and E-64, but no NP-40 (hypotonic, no-detergent extraction buffer). The cells were then transferred to a Dounce homogenizer and homogenized on ice until the cells (but not the nuclei) were broken. The material was centrifuged at 15,000 g for 30 min and the supernate saved as a no-detergent cell extract. The remaining pellet was then extracted in the NP-40-containing extraction buffer as described above.

Immunoprecipitation

Immunoprecipitations were done in several ways. For experiments with radiolabeled cells a 100- μ l aliquot of cell extract was mixed with 100 μ l mAb supernate at 4°C. For experiments presented in Figs. 2 and 6, 300 μ l of extract and 300 μ l antibody supernate were used. After 30 min, 50 μ l packed anti-mouse IgG-Sepharose (Organon Teknika Corp-Cappell, West Chester, PA) was added and mixing was continued for another 30 min. For all other immunoprecipitations, cell extracts were mixed with a mAb covalently coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech Inc., Piscataway, NJ) at a ratio of 5 ml cell extract per ml mAb-Sepharose. The mAbs were purified from conditioned medium by 50% ammonium sulfate precipitation followed by affinity chromatography using immobilized anti-mouse IgG (Organon Teknika Corp-Cappell, West Chester, PA). mAbs and normal mouse IgG were coupled to Sepharose at a concentration of 3 mg antibody/ml CNBr-activated Sepharose 4B according to the manufacturer's instructions. The IgG-Sepharose was cycled according to the manufacturer's instructions and was blocked with 3% BSA in 10 mM Tris-HCl, pH 7.5, containing 0.5% NP-40, and 100 μ M Ca²⁺ for a minimum of 30 min mixing at room temperature prior to use. Subsequently, the cell extract, which was absorbed first against inactivated Sepharose, and IgG-Sepharose were mixed at 4°C for 1.5 h. All immunoprecipitates were washed five times with a 10-fold excess volume of either: 10 mM Tris-HCl, pH 7.5, containing 0.5% NP-40, and 100 μ M Ca²⁺ (low stringency wash buffer); low stringency buffer with 0.3 M NaCl (salt wash buffer); or 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl, 1% Triton X-100, 0.5% deoxycholate, and 0.1% SDS (high stringency wash buffer). For all immunoprecipitations, the Sepharose was treated with Laemmli sample buffer to elute the bound proteins, which were then resolved by SDS-PAGE.

Electrophoresis and Western Immunoblotting

SDS-PAGE was done according to the procedure of Laemmli (1970) using a 7 or 8% resolving gel and a 3.5% stacking gel. Materials were from Bio-Rad Labs. (Richmond, CA). Molecular mass markers (Sigma) included myosin (205 kD), β -galactosidase (116 kD), phosphorylase b (97 kD), BSA (66 kD), ovalbumin (43 kD), and carbonic anhydrase (30 kD). Autoradiography was performed as previously described (Wheelock, 1990). Radiolabeled molecular mass standards were from GIBCO BRL (Gaithersburg, MD). For Western immunoblotting the SDS-PAGE-resolved proteins were transferred electrophoretically to nitrocellulose, the nitrocellulose was blocked with 3–4% BSA, and the various proteins of interest were detected by specific polyclonal or mAbs followed by appropriate species-specific and antibody isotype-specific alkaline phosphatase-conjugated antibodies (Promega, Madison, WI) and nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates (NBT/BCIP) as described (Knudsen and Wheelock, 1992).

Immunofluorescence Light Microscopy

For immunofluorescence light microscopy, cells were grown on glass coverslips and fixed with Histochoice (Amresco, Solon, OH) following the manufacturer's instructions. The cells were blocked with 10% goat serum and 0.1 M glycine in PBS for 20 min and subsequently exposed for 1 h to antibodies specific for the proteins of interest. Primary antibodies were detected by species-specific secondary antibodies conjugated to either rhodamine or fluorescein (Jackson ImmunoResearch Labs., Inc., West Grove, PA). Fluorescence was detected with a Zeiss Axiophot (Carl Zeiss, Inc., Thornwood, NY) microscope equipped with epifluorescence and filters appropriate for visualizing rhodamine or fluorescein and photographed using Kodak T-MAX 3200 or Kodak Ektachrome 320T film. All pictures were taken using either the 40 or 100 \times objective.

Results

Normal Human Fibroblasts Express an N-Cadherin/Catenin Complex

Fibroblasts were previously reported to express cadherins, with chicken fibroblasts having N-cadherin (Hirano et al., 1987) both in vitro and in vivo and the 3Y1 normal rat fibroblast cell line expressing P-cadherin (Itoh et al., 1991). Both cadherins were shown to mediate Ca^{2+} -dependent fibroblast aggregation in vitro, indicating the cadherins are functionally active in fibroblasts. Here we show that human fibroblasts interact with one another and express an N-cadherin/catenin complex.

Normal human WI-38 fibroblasts show a variety of cell-cell interactions detectable at the light microscope level. Some involve stretches of continuous membrane interaction, whereas others involve finger-like projections from one cell onto another. In addition, like Heaysman and Pegrum (1973), we observed structures resembling cell-cell adherens junctions at the ultrastructural level (data not shown). To determine if the WI-38 cells express a cadherin, we employed immunoblot analysis of NP-40 cell extracts. Fig. 1 shows that WI-38 fibroblasts express a 135-kD protein (lane 4) detected by an anti-N-cadherin mAb (13A9). The 13A9 monoclonal antibody is specific for N-cadherin. It did not recognize E-cadherin expressed by JAR epithelial cells (lane 3), nor did it recognize P-cadherin in A431 epithelial cells (data not shown).

Immunoblotting with a monoclonal antibody specific for E-cadherin detected no E-cadherin in the extract of the WI-38 fibroblasts (lane 2), whereas the antibody readily detected E-cadherin in the JAR epithelial cell extract (lane 1). In addition, P-cadherin was not detected in WI-38 cells (data not shown). Moreover, only the 135-kD band

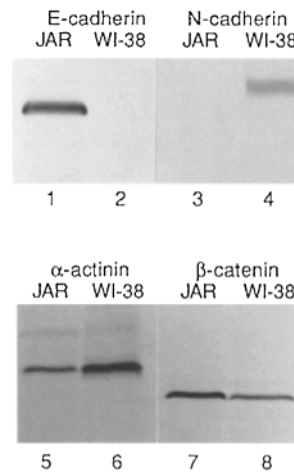


Figure 1. Comparison of the expression of cell-cell adhesion molecules in WI-38 fibroblasts and JAR epithelial cells. NP-40 extracts of WI-38 fibroblasts (lanes 2, 4, 6, and 8) and JAR epithelial cells (lanes 1, 3, 5, and 7) were resolved on a 7% SDS-polyacrylamide gel, transblotted to nitrocellulose, and probed with specific mAbs, followed by species-specific, alkaline phosphatase-conjugated secondary antibodies and NBT/BCIP. 10 μ g total protein was loaded in each lane. Lanes 1 and 2 were immunoblotted with anti-E-cadherin (E9), lanes 3 and 4 with anti-N-cadherin (13A9), lanes 5 and 6 with anti- α -actinin (E2), and lanes 7 and 8 with anti- β -catenin (9F2). Markers for the 116- and 97-kD molecular mass standards are indicated at the right.

was detected using a polyclonal anti-(pan) cadherin antiserum. To confirm that the cadherin expressed by the WI-38 cells was N-cadherin, a normal human fibroblast cDNA library was screened with a cDNA probe encoding human N-cadherin. The clones were sequenced and shown to be identical to the reported human N-cadherin sequence (Reid and Hemperly, 1990). Together, the data indicated that N-cadherin is the only classic cadherin expressed by WI-38 fibroblasts. N-cadherin was also detected in normal human fibroblasts isolated from juvenile foreskin and adult lung and skin cells obtained from Coriell Institute for Medical Research (data not shown). In Fig. 1 an identical amount of protein from JAR epithelial cell and WI-38 fibroblast NP-40 cell extract was loaded in each lane. Comparing the anti-E-cadherin signal in the JAR epithelial cell extract (lane 1) to the anti-N-cadherin signal in WI-38 extract (lane 4), it appears as if the epithelial cells have more cadherin. This might be expected considering epithelial cells form more extensive and stable cell-cell contacts than do fibroblasts. Of course, the comparison is complicated by the fact that the anti-E- and anti-N-cadherin antibodies may have different affinities for their respective antigens. As expected, both cell types express α -actinin (lanes 5 and 6). It is to be noted that the WI-38 cells express more α -actinin than the JAR cells. This is likely due to the more extensive microfilament organization exhibited by the fibroblasts. Both cell types also express α -catenin (see Fig. 2 for W138 cells and Wheelock and Knudsen, 1991 for JAR cells), β -catenin (Fig. 1, lanes 7 and 8), and plakoglobin (see Fig. 2 for W138 cells and Knudsen and Wheelock, 1992 for JAR cells). Somewhat lower levels of catenin expression were observed in the WI-38 fibroblasts compared to the JAR epithelial cells (Fig. 1, compare lanes 7 and 8), consistent with the notion that the fibroblasts express a somewhat lower level of the cadherin/catenin cell-cell adhesion complex compared to epithelial cells.

As shown for other cadherin-expressing cells, N-cadherin forms a complex with the catenins in WI-38 fibroblasts. This multiprotein complex can be captured by im-

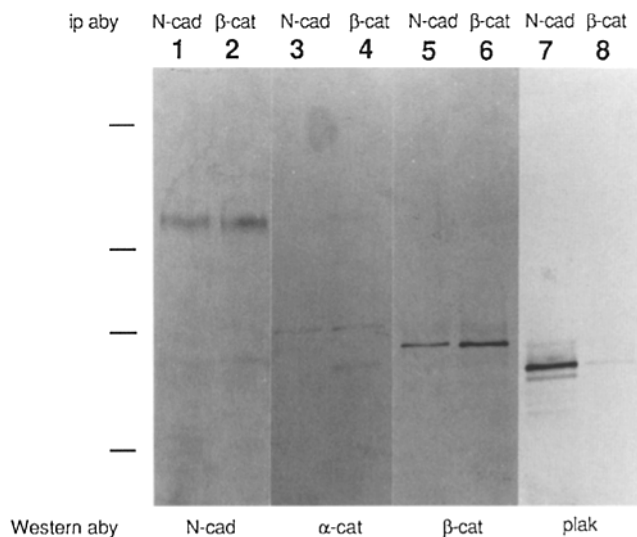


Figure 2. Fibroblasts contain an N-cadherin/catenin complex. WI-38 NP-40 extract was immunoprecipitated with monoclonal anti-N-cadherin (13A9; lanes 1, 3, 5, and 7) or anti-β-catenin (9F2, lanes 2, 4, 6, and 8). The precipitates were washed with stringent wash buffer. The proteins were resolved on a 7% SDS-polyacrylamide gel, transblotted to nitrocellulose, and probed with specific antibodies, followed by species-specific, alkaline phosphatase-conjugated second antibodies and NBT/BCIP substrates. Lanes 1 and 2 were immunoblotted with anti-N-cadherin (13A9), lanes 3 and 4 with anti-α-catenin (1G5), lanes 5 and 6 with anti-β-catenin (9F2), and lanes 7 and 8 with anti-plakoglobin (15F11). The 86-kD band in lane 4 is an α-catenin breakdown product. The immunoprecipitating antibodies (*ip aby*) are noted along the top of the figure and the immunoblotting antibodies (*Western aby*) are noted along the bottom of the figure. The lines at the left indicate the molecular mass standards at 205, 116, 97, and 66 kD. Note that the β-catenin immunoprecipitation contains relatively more β-catenin and less plakoglobin than does the N-cadherin immunoprecipitation, suggesting the existence of separate complexes containing predominantly either β-catenin or plakoglobin.

munoprecipitating one component, e.g., the cadherin or a catenin. In Fig. 2 we present the results of immunoblot analysis of anti-N-cadherin and anti-β-catenin immunoprecipitates of WI-38 NP-40 extract. The antibody used for the immunoprecipitation (*ip aby*) is indicated at the top of the figure, whereas the antibody used for Western immunoblot analysis (*Western aby*) of the immunoprecipitate is indicated at the bottom of the figure. When N-cadherin was immunoprecipitated, the following proteins were detected in the immunoprecipitate: N-cadherin (lane 1), α-catenin (lane 3), β-catenin (lane 5), and plakoglobin (lane 7). When β-catenin was immunoprecipitated, the following proteins were detected in the immunoprecipitate: N-cadherin (lane 2), α-catenin (lane 4), and β-catenin (lane 6). Only a trace amount of plakoglobin was detected in the β-catenin immunoprecipitate (lane 8), indicating that the N-cadherin/catenin complex contains predominantly either β-catenin or plakoglobin.

In addition to forming a complex with catenins, N-cadherin colocalizes with catenins in WI-38 fibroblasts. Fig. 3 shows immunofluorescence colocalization. *A* and *B* depict the same cells stained for N-cadherin and β-catenin, re-

spectively. The phase contrast image of these cells is shown in *C*. *D* and *E* show the same cells stained for N-cadherin and α-catenin, respectively, while *F* shows the phase contrast image. Note the extensive overlap of N-cadherin and catenin staining, indicating that N-cadherin complexes with catenins at the plasma membrane and is particularly prominent at regions of cell-cell contact. This extensive colocalization of cadherin and catenins is similar to that observed previously by us in E-cadherin-expressing epithelial cells and N-cadherin-expressing cardiomyocytes (Knudsen and Wheelock, 1992).

Together, our immunoprecipitation and colocalization results indicate that N-cadherin complexes with catenins in WI-38 fibroblasts and that the complex includes N-cadherin, either β-catenin or plakoglobin, and α-catenin.

In Fibroblasts the N-Cadherin/Catenin Complex Colocalizes Extensively with α-Actinin, but Not Vinculin

It has been well documented that in cell types such as epithelial cells and cardiac myocytes, vinculin and α-actinin colocalize extensively with the cadherin/catenin complex at cell-cell adherens junctions. In addition, vinculin and α-actinin are also found along with integrins at cell-matrix adhesion contacts. These two cytoskeletal proteins bind to each other and also to actin. In Fig. 4 we show colocalization of E-cadherin with α-actinin (*A* and *B*) and with vinculin (*C* and *D*) in the JAR epithelial cells. Note the extensive overlap of the cadherin with vinculin in the JAR epithelial cells; this was not observed in the WI-38 fibroblasts.

In fibroblasts, both vinculin and α-actinin are highly expressed and both proteins are associated with cell-matrix contacts, which complicates the staining pattern. However, upon careful examination of the WI-38 cells, we saw little colocalization between α-catenin, as a marker of the cadherin/catenin complex, and vinculin. In Fig. 5 *A* the same cells were stained for α-catenin (*A*) and vinculin (*B*). In some places the individual stainings crossed each other going different directions, but only rarely did the vinculin staining mimic precisely the α-catenin staining. In contrast, we observed extensive colocalization between the cadherin/catenin complex and α-actinin in the WI-38 cells. In Fig. 5 *A* the same cells are stained for α-catenin (*C*) and α-actinin (*D*). Arrows point to a region of cell-cell contact where there is extensive overlap of the α-catenin and α-actinin staining. To further demonstrate the difference in colocalization of α-actinin and vinculin with α-catenin, we photographed the cells using double exposure of color film (Fig. 5 *B*). Polyclonal anti-α-catenin was detected with rhodamine-labeled anti-rabbit IgG (*red*) and monoclonal anti-α-actinin or anti-vinculin was detected with FITC-labeled anti-mouse IgG (*green*). In *A* the overlap of vinculin and α-catenin staining at regions of cell-cell contact (*arrow*) is minimal. In contrast, as shown in *B*, the overlap of α-actinin and α-catenin staining at regions of cell-cell contact (*arrow*) is extensive, as indicated by the yellow color.

α-Actinin, but Not Vinculin, Coimmunoprecipitates with the Cadherin/Catenin Complex

The colocalization of the cadherin/catenin complex with

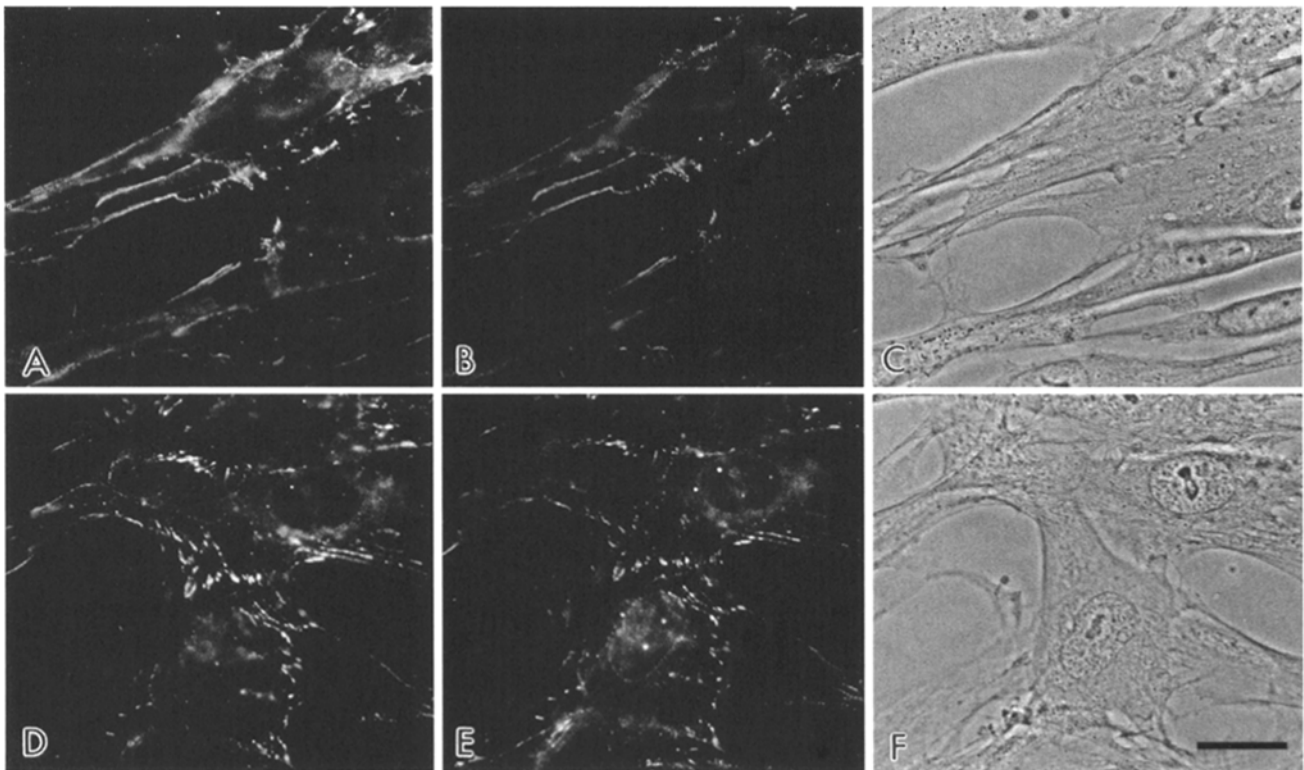


Figure 3. N-Cadherin and the catenins colocalize at sites of cell-cell contact in fibroblasts. WI-38 cells were grown on glass coverslips, washed thoroughly, fixed with Histochoice, and exposed to monoclonal anti-N-cadherin (13A9) together with either polyclonal anti- β -catenin (A–C) or polyclonal anti- α -catenin (D–F). The polyclonal antibodies were detected with rhodamine-labeled species-specific goat anti-rabbit IgG and the monoclonal antibody was detected with FITC-conjugated species-specific goat anti-mouse IgG. There was no cross-reactivity between the species-specific second antibodies; nor was there any cross-detection of rhodamine in the FITC channel or FITC in the rhodamine channel. A and B compare the immunofluorescence localization of N-cadherin (A) with that of β -catenin (B). D and E compare immunofluorescence localization of N-cadherin (D) with that of α -catenin (E). Note the two different types of cell-cell contacts shown in A vs. D. C and F show phase microscopy of the cells shown in A and B (D and E), respectively. Bar, 10 μ m.

α -actinin at the plasma membrane of cells led us to consider the possibility that α -actinin links the cadherin/catenin complex to the actin cytoskeleton. To investigate this possibility we isolated the catenins and the cadherin/catenin complex by immunoprecipitation and looked for the presence of α -actinin in the precipitates by immunoblot analysis. Previously, we and others identified the catenins by this method, but failed to detect any α -actinin. However, we scaled up the immunoprecipitations to maximize the chance of detecting α -actinin if it bound to only a fraction of the N-cadherin/catenin complex in cells, or if its interaction with the complex only partially survived the extraction and immunoprecipitation procedures. To make this approach more experimentally feasible, we employed HeLa cells, which grow more vigorously than WI-38 fibroblasts. Like WI-38 fibroblasts, HeLa cells express the N-cadherin/catenin complex (Herrenknecht et al., 1991; data presented here), but not E- or P-cadherin (data not shown). Moreover, we observed that the N-cadherin/catenin complex in HeLa cells, like that in WI-38 cells, colocalized extensively with α -actinin, but not vinculin (data not shown).

Initially the HeLa cells were extracted with nonionic detergent (NP-40) and the extract exposed to Sepharose 4B-conjugated anti- α -catenin (1G5). The immunoprecipitate was washed under low stringency conditions (10 mM Tris-

HCl, pH 7.5, 0.5% NP-40, 100 μ M Ca^{2+}) to maximize the chance that even low affinity protein-protein interactions would survive. Fig. 6 A shows that under these conditions α -actinin (lane 4), but not vinculin (lane 5), was detected by immunoblot analysis in the immunoprecipitate. The minor 105-kD band seen in lane 5 of Fig. 6 A is not vinculin; rather, it is due to the second antibody and the long development time used to maximize the chance of detecting vinculin. Vinculin has a molecular mass of 120 kD and migrates above the 116-kD molecular mass marker. Fig. 6 B shows that both vinculin and α -actinin were present and readily detectable by immunoblot analysis in the whole NP-40 cell extract. As expected, N-cadherin (lane 1), β -catenin (lane 2), and plakoglobin (not shown) were also present in the immunoprecipitate, indicating the formation of N-cadherin/catenin complexes. We found that some, but not all, of the α -actinin, could be released from the immunoprecipitate by adding 0.3 M NaCl to the low stringency wash buffer (salt wash). It is of interest that a sensitivity to salt was previously observed for the interaction between α -actinin and the β_1 integrin subunit (Otey et al., 1990). However, no additional α -actinin was released from the immunoprecipitate by washing it with a stringent wash buffer containing 0.15 M NaCl, Triton X-100, 0.5% deoxycholate, and 0.1% SDS.

Immunoprecipitations of metabolically ^{35}S -radiolabeled

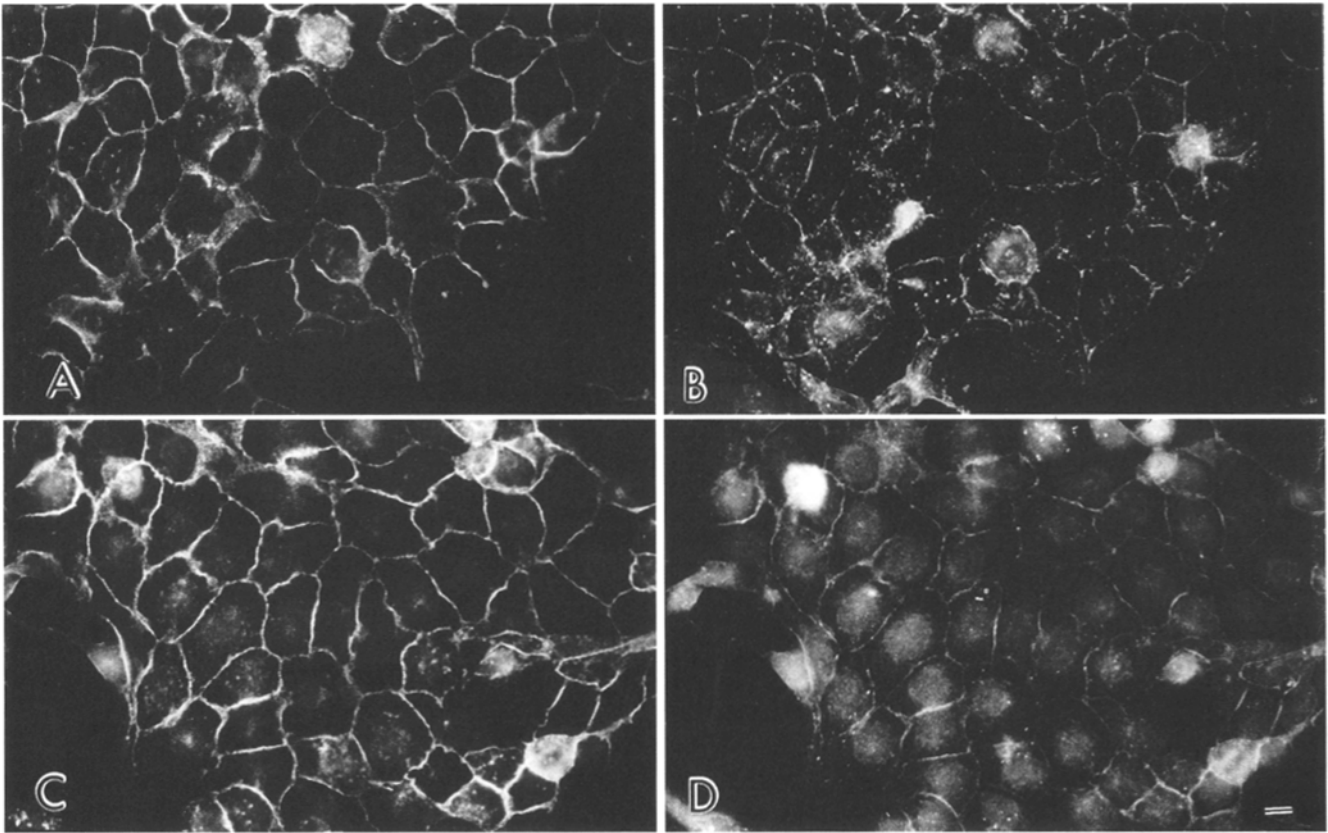


Figure 4. E-Cadherin colocalizes with α -actinin and vinculin in epithelial cells. JAR cells were grown on glass coverslips, washed thoroughly, fixed with Histochoice, and exposed to polyclonal anti-E-cadherin (A and C) together with monoclonal anti- α -actinin (B) or anti-vinculin (D). mAbs to α -actinin (E2) and vinculin (*hVIN-1*) were detected with species-specific FITC-conjugated goat anti-mouse IgG. Polyclonal anti-E-cadherin was detected with rhodamine-conjugated anti-rabbit IgG. There was no cross-reactivity between the second antibodies; nor was there any cross-detection of rhodamine in the FITC channel or FITC in the rhodamine channel.

HeLa cells washed with the low stringency buffer did not show a general increase in proteins present in the immunoprecipitates. This result suggested that α -actinin detected in Fig. 6 bound specifically to the immunoprecipitate. Fig. 7 shows the autoradiogram of the proteins present in anti-N-cadherin (13A9, lane 1) and anti- α -catenin (1G5, lane 2) immunoprecipitations compared to the non-immune mouse IgG control (lane 3). An anti- β -catenin (9F2) immunoprecipitate looked almost identical to the anti-N-cadherin immunoprecipitate, except that, as expected, only a minor amount of plakoglobin was present (data not shown). Bands with molecular mass corresponding to N-cadherin (which is poorly radiolabeled), α -catenin, β -catenin, and plakoglobin were all present in both immune precipitates. The α -catenin band in lane 2 is thicker than that in lane 1, but appears to contain only one band. There is more α -catenin in lane 2 because the anti- α -catenin antibody bound both N-cadherin-associated and N-cadherin-free α -catenin. In contrast, the anti-N-cadherin (lane 1) immunoprecipitated only the α -catenin associated with N-cadherin. The band ~ 86 kD present in the α -catenin immunoprecipitate (lane 2), but not the N-cadherin immunoprecipitate (lane 1), likely represents an α -catenin breakdown product that binds to the 1G5 antibody but does not bind to the N-cadherin-containing com-

plex. Other bands in these low-stringently washed immune precipitates (lanes 1 and 2) are common to the low-stringently washed nonimmune precipitate (lane 3) and presumably represent nonspecifically bound proteins. α -actinin cannot be discerned in the nonstringently washed, radiolabeled immunoprecipitate, perhaps because: its molecular mass (100 kD) is so similar to that of α -catenin (102 kD); like N-cadherin, it did not label well; or there is too little to be detected. Note that, except for those shown in Figs. 6 and 7, all other immunoprecipitates presented here were washed with the stringent buffer.

The colocalization of α -actinin with α -catenin at the plasma membrane shown in Fig. 5 suggested that α -actinin interacts with the cadherin/catenin complex. To gather support for this we used two approaches. In one approach, we immunoprecipitated the N-cadherin and looked for α -actinin in the immunoprecipitate; in this case the anti-N-cadherin antibody (13A9) immunoprecipitated N-cadherin and only those catenins bound to the cadherin. In another approach, we separated and immunoprecipitated both soluble and particulate pools of catenins. To generate these two pools of catenins, we first lysed the HeLa cells in a hypotonic buffer in the absence of detergent. This no-detergent extract contained α -catenin, β -catenin, plakoglobin, α -actinin, and actin, but no N-cadherin (data not

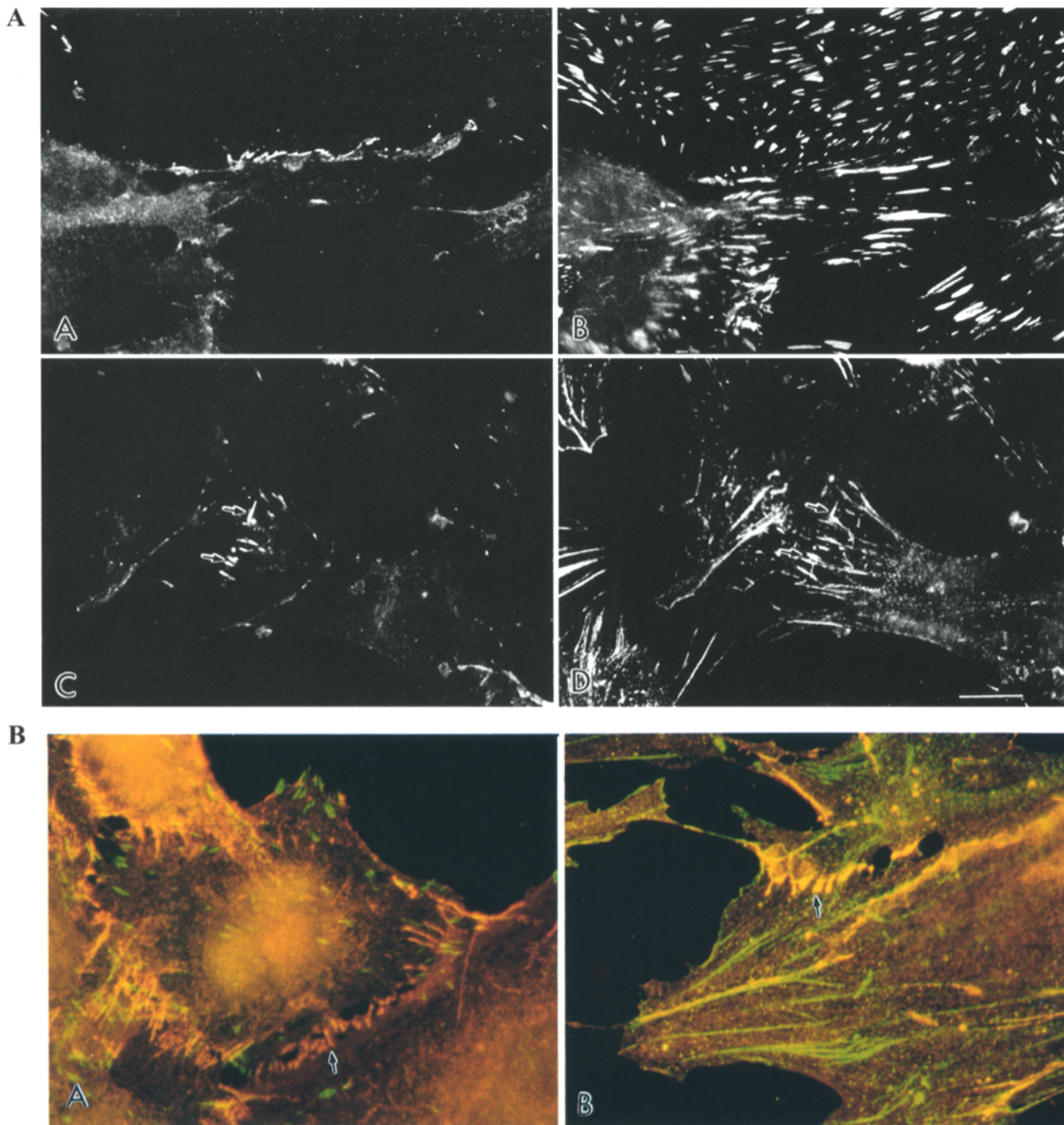


Figure 5. α -Catenin colocalizes with α -actinin, but not vinculin, in fibroblasts. (A) WI-38 cells were grown on glass coverslips, washed thoroughly, fixed with Histochoice, and exposed to polyclonal anti- α -catenin (A and C) together with either monoclonal anti-vinculin (B) or anti- α -actinin (D). mAbs to vinculin (*hVIN-1*) and α -actinin (E2) were detected with species-specific FITC-conjugated goat anti-mouse IgG. Polyclonal anti- α -catenin was detected with rhodamine-conjugated anti-rabbit IgG. There was no cross-reactivity between the second antibodies; nor was there any cross-detection of rhodamine in the FITC channel or FITC in the rhodamine channel. A and B show that α -catenin (A) does not colocalize extensively with vinculin (B), whereas C and D show extensive colocalization of α -catenin (C) with α -actinin (D). Arrows in C and D point out regions of cell-cell contact that are intensely stained for both α -catenin and α -actinin. (B) Presentation of the above experiment in color further points out the colocalization of α -catenin (red) with α -actinin (green in B), but not vinculin (green in A). Regions where the α -catenin and α -actinin colocalize are seen as yellow (pointed out by an arrow in B). Extensive yellow at regions of cell-cell contact is not observed in A; a region of cell-cell contact stained by anti- α -catenin (red) but not vinculin (green) is pointed out by an arrow. Bar, 10 μ m.

shown). The insoluble material was then extracted with NP-40. This extract contained N-cadherin and all three catenins, in addition to actin and α -actinin.

In Fig. 8 we show by immunoblot analysis that α -actinin

was present in both anti- α -catenin (1G5, lane 2) and anti-N-cadherin (13A9, lane 4) immunoprecipitates of the detergent extract of hypotonically shocked HeLa cells, but not in the nonimmune control (lane 6). Note that in lanes

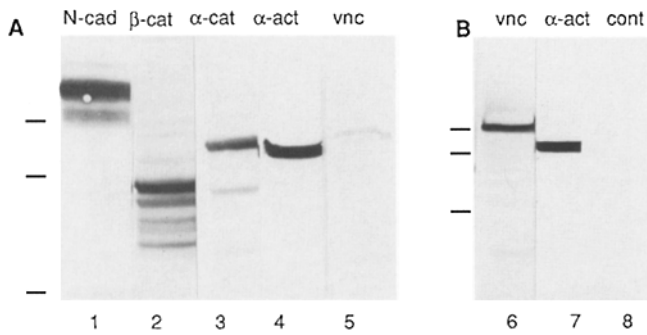


Figure 6. α -Actinin, but not vinculin, coimmunoprecipitates with α -catenin from extracts of N-cadherin-expressing HeLa cells. (A) Confluent monolayers of HeLa cells were extracted in Tris/NP-40 buffer containing 100 μ M Ca^{2+} and protease inhibitors as described in Materials and Methods. The extract was immunoprecipitated with Sepharose-conjugated anti- α -catenin (1G5) as described in Materials and Methods. The precipitate was washed with the low stringency buffer and eluted with Laemmli sample buffer. The proteins were resolved by SDS-PAGE using a 7% acrylamide gel, transferred electrophoretically to nitrocellulose, and probed with the designated mouse mAbs followed by alkaline phosphatase-conjugated anti-mouse IgG (lanes 1–3 and 5) or IgM (lane 4) and NBT/BCIP substrates. Lane 1, anti-N-cadherin (13A9); lane 2, anti- β -catenin (9F2); lane 3, anti- α -catenin (1G5); lane 4, anti- α -actinin (BM-75.2); lane 5, anti-vinculin (VIN-11-5). Note that α -actinin, but not vinculin, is detected in the 1G5 immunoprecipitate. Note also the slight, but discernible, difference in the molecular mass of α -catenin (102 kD) and α -actinin (100 kD). The lines at the left of A indicate the molecular mass standards at 116, 97, and 66 kD. (B) HeLa NP-40 cell extract was resolved by SDS-PAGE using a 7% acrylamide gel. The proteins were transferred electrophoretically to nitrocellulose and probed with the designated mouse mAbs followed by alkaline phosphatase-conjugated anti-mouse IgG or IgM. Lane 6, anti-vinculin (VIN-11-5); lane 7, anti- α -actinin (BM-75.2); lane 8 is a control stained with second anti-IgM antibody alone. Note the cell extract contains a large amount of vinculin, although none coimmunoprecipitated with the cadherin/catenin complex. The lines at the left of B indicate the 116-, 97-, and 66-kD molecular mass standards.

2, 4, and 6 the thin black line near the 68-kD molecular mass marker designates the location where the nitrocellulose was cut horizontally before being exposed to the blotting antibody. Only the top portions were exposed to anti- α -actinin, whereas the bottom portions were exposed to anti- β -actin. As expected, the anti- α -catenin and anti-N-cadherin immunoprecipitates contained β -catenin (lanes 3 and 5, respectively), indicating the presence of cadherin/catenin complexes. The nonimmune control had no β -catenin (lane 7). Because α -actinin is known to bind actin, we also immunoblotted the immunoprecipitates with a high-titer anti-actin antibody (bottom portions of lanes 2, 4 and 6). Only a trace amount of actin was detected in the immune precipitates (lanes 2 and 4, bottom) compared to the nonimmune control (lane 6, bottom). Together, the results suggest strongly that α -actinin interacts with the N-cadherin/catenin complex and likely does so through a direct interaction not involving actin.

Fig. 9 A shows that α -actinin also coimmunoprecipitates with the material captured from the NP-40 extract of hy-

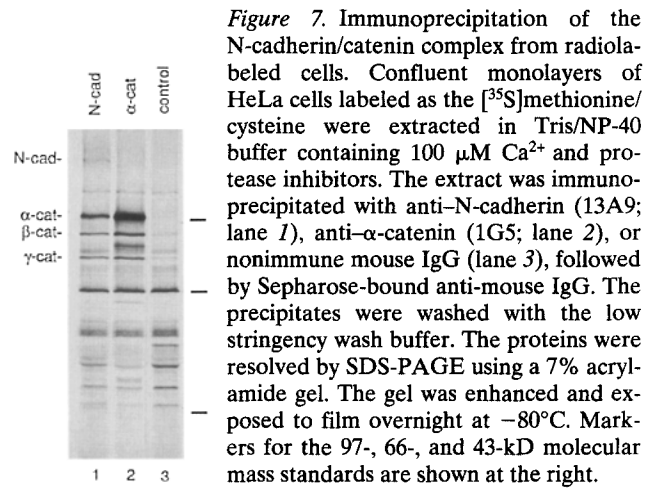


Figure 7. Immunoprecipitation of the N-cadherin/catenin complex from radiolabeled cells. Confluent monolayers of HeLa cells labeled as the [^{35}S]methionine/cysteine were extracted in Tris/NP-40 buffer containing 100 μ M Ca^{2+} and protease inhibitors. The extract was immunoprecipitated with anti-N-cadherin (13A9; lane 1), anti- α -catenin (1G5; lane 2), or nonimmune mouse IgG (lane 3), followed by Sepharose-bound anti-mouse IgG. The precipitates were washed with the low stringency wash buffer. The proteins were resolved by SDS-PAGE using a 7% acrylamide gel. The gel was enhanced and exposed to film overnight at -80°C . Markers for the 97-, 66-, and 43-kD molecular mass standards are shown at the right.

potentially shocked HeLa cells by the 10C4 antibody that recognizes β -catenin and plakoglobin (lane 3). This is compared to a similar anti- α -catenin immunoprecipitate (lane 1). As expected, N-cadherin was detected in both immunoprecipitates (lanes 2 and 4), indicating the presence of complexes. Neither α -actinin nor N-cadherin was detected in the nonimmune control precipitate (lanes 5 and 6, respectively). These results support an interaction of α -actinin with the N-cadherin/catenin complex. In contrast, Fig. 9 B shows that α -actinin (lane 1) did not coimmunoprecipitate with soluble catenins from the hypotonic,

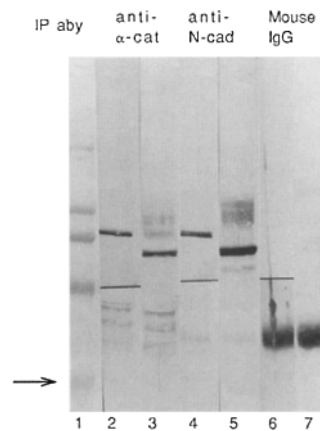


Figure 8. α -Actinin coimmunoprecipitates with the N-cadherin/catenin complex isolated from HeLa cells. HeLa cells were Dounce homogenized in hypotonic buffer in the absence of detergent and centrifuged to remove the insoluble material. The resulting pellet was extracted with NP-40 and immunoprecipitated with Sepharose-conjugated anti- α -catenin (1G5; lanes 2 and 3), anti-N-cadherin (13A9; lanes 4 and 5), or nonimmune mouse IgG (lanes 6 and 7), as described

in Materials and Methods. The precipitates were washed thoroughly with stringent buffer, eluted with Laemmli sample buffer, and resolved by SDS-PAGE. The proteins were transferred electrophoretically to nitrocellulose for Western immunoblotting. Lanes 2, 4, and 6 were cut horizontally near the 66-kD molecular mass marker as indicated by the thin black line. The tops of lanes 2, 4, and 6 were blotted with anti- α -actinin (BM-75.2), whereas the bottoms were blotted with anti- β -actin (AC-15). Lanes 3, 5, and 7 were blotted with anti- β -catenin (9F2). The mouse mAbs were detected with alkaline phosphatase-conjugated anti-mouse IgG (for AC-15 and 9F2) or IgM (for BM-75.2) and NBT/BCIP substrates. The immunoprecipitating antibody (IP *aby*) is noted at the top of the figure. The molecular mass standards at 205, 116, 97, 66, and 43 kD are shown in lane 1. The broad, dark bands in lanes 6 and 7 indicate mouse IgG heavy chain recognized by the secondary antibody. The arrow indicates the position of actin.

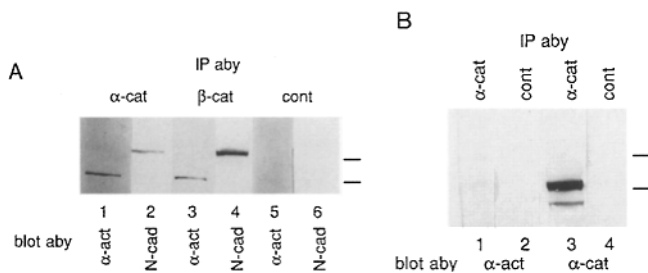


Figure 9. α -Actinin associates with the particulate, but not with the soluble, pool of catenins. (A) HeLa cells were Dounce homogenized in hypotonic buffer in the absence of detergent and centrifuged to remove insoluble material. Both the pellet and supernate were saved. The pellet was subsequently extracted with NP-40 and immunoprecipitated with Sepharose-conjugated anti- α -catenin (1G5), anti- β -catenin (10C4), and nonimmune mouse IgG, as described in Materials and Methods. The immunoprecipitates were washed with stringent buffer, eluted with Laemmli sample buffer, and resolved by SDS-PAGE. The proteins were transferred electrophoretically to nitrocellulose and probed with the designated mouse mAbs, followed by alkaline phosphatase-conjugated anti-mouse IgG (lanes 2, 4, and 6) or IgM (lanes 1, 3, and 5) and NBT/BCIP substrates. Lanes 1, 3, and 5, anti- α -actinin (BM 75.2). Lanes 2, 4, and 6, anti-N-cadherin (13A9). The immunoprecipitating antibody (*IP aby*) is noted at the top of the figure and the immunoblotting antibody (*blot aby*) at the bottom. Markers for the 116- and 97-kD molecular mass standards are shown at the right. (B) The no-detergent extract (i.e., supernate after centrifuging the Dounced cells as described in A above) was immunoprecipitated with Sepharose-conjugated anti- α -catenin (1G5) and control mouse IgG as described in Materials and Methods. The immunoprecipitates were washed with stringent wash buffer, eluted with sample buffer, and resolved by SDS-PAGE. The proteins were transferred electrophoretically to nitrocellulose and probed with the designated mouse mAbs, followed by alkaline phosphatase-conjugated anti-mouse IgG or IgM and NBT/BCIP substrates. Lanes 1 and 2, anti- α -actinin (BM-75.2). Lanes 3 and 4, anti- α -catenin (1G5). Note the absence of α -actinin in both the immune and nonimmune precipitates. Markers for the 116- and 97-kD molecular mass standards are shown at the right.

no-detergent extract of HeLa cells; nor was it present in the nonimmune control (lane 2). α -catenin was readily detected in the immunoprecipitate (lane 3), but not in the nonimmune control (lane 4), indicating that the immunoprecipitation was successful. β -catenin and plakoglobin were also present in the immunoprecipitate, presumably bound to α -catenin (data not shown). These data suggested that α -actinin does not bind to soluble catenins. However, it is possible that an interaction of α -actinin with soluble catenins is less stable than that with the complex and therefore did not survive our extract/immunoprecipitation conditions.

α -Actinin Coimmunoprecipitates with Catenins Isolated from E-Cadherin-expressing Cells, but Only When α -Catenin Is Present

The questions arose as to whether or not α -actinin associated with catenins isolated from E-cadherin-expressing cells and also whether it associated with α -catenin or β -catenin. To address these questions we turned to two ep-

ithelial cell lines: JAR, which expresses a normal E-cadherin/catenin complex, and PC9, which expresses an E-cadherin/catenin complex missing α -catenin (Shimoyama et al., 1992; Watabe et al., 1994). The PC9 and JAR cells both express α -actinin at levels similar to that of HeLa cells (data not shown).

Fig. 10 shows that α -actinin also coimmunoprecipitated with α -catenin isolated from the JAR human epithelial cell line. The JAR NP-40 extract was immunoprecipitated with the IG5 anti- α -catenin antibody and immunoblotted for α -actinin. The figure shows that α -actinin was detected in the immune precipitate (lane 1), but not in the nonimmune precipitate (lane 2). E-cadherin, β -catenin, and α -catenin were as present as expected (data not shown). It is interesting to note that our E2 anti-(human) α -actinin monoclonal antibody resulted from a fusion in which the rat was injected with E-cadherin/catenin complex isolated from JAR cells using the E9 anti-(human)E-cadherin antibody (Wheelock, 1990), suggesting α -actinin also associates with the E-cadherin/catenin complex. No vinculin was detected in the α -catenin immunoprecipitate (data not shown), even though it colocalized with the E-cadherin in the JAR cells (see Fig. 4).

We then immunoprecipitated the β -catenin from an NP-40 extract of the α -catenin-free PC9 cells and examined the precipitate for the presence of α -actinin. To do this we used the 10C4 monoclonal antibody, which recognizes both β -catenin and plakoglobin. Fig. 11 shows the immunoblot analysis of the immunoprecipitate. As expected, E-cadherin was present in the immunoprecipitate (lane 3), but not in the nonimmune control (lane 1), indicating the presence of E-cadherin/ β -catenin (or plakoglobin) complexes. However, in contrast to the JAR immunoprecipitate (Fig. 10), α -actinin was not detected in the PC9 immune precipitate (lane 4); nor was it in the nonimmune precipitate (lane 2). These data indicated that in the absence of α -catenin, α -actinin does not associate with β -catenin (or plakoglobin) or with E-cadherin/ β -catenin (or plakoglobin) complexes.

Discussion

We present evidence in this paper that α -actinin interacts specifically with cadherin/catenin complexes and, furthermore, that this interaction depends upon the presence of

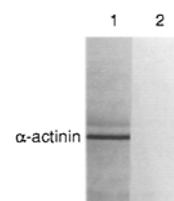


Figure 10. α -Actinin coimmunoprecipitates with α -catenin from the extract of E-cadherin-expressing JAR cells. Confluent monolayers of JAR cells were extracted in Tris/NP-40 buffer containing 100 μ M Ca^{2+} and protease inhibitors as described in Materials and Methods. The extract was immunoprecipitated with Sepharose-conjugated anti- α -catenin (1G5; lane 1) or control beads containing mouse IgG (lane 2) as described in Materials and Methods. The precipitates were washed with stringent buffer and resolved by SDS-PAGE using a 7% acrylamide gel. The proteins were transferred electrophoretically to nitrocellulose and probed with anti- α -actinin (BM-75.2) followed by alkaline phosphatase-conjugated anti-mouse IgM and NBT/BCIP substrates. Markers for the 116- and 97-kD molecular mass standards are shown at the right.

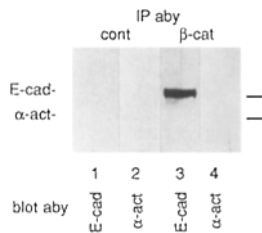


Figure 11. α -Actinin does not coimmunoprecipitate with β -catenin from extracts of PC9 cells that express E-cadherin but are missing α -catenin. Confluent monolayers of PC9 cells were extracted in Tris/NP-40 buffer containing 100 μ M Ca^{2+} and protease inhibitors as described in Materials and Methods.

The extract was immunoprecipitated with Sepharose-conjugated anti- β -catenin (10C4; lanes 3 and 4) or control beads with coupled mouse IgG (lanes 1 and 2), as described in Materials and Methods. The precipitates were washed with stringent buffer, eluted with Laemmli buffer, and resolved by SDS-PAGE using a 7% acrylamide gel. The proteins were transferred electrophoretically to nitrocellulose and probed with anti-E-cadherin (E-9; lanes 1 and 3) or anti- α -actinin (BM-75.2; lanes 2 and 4) followed by alkaline phosphatase-conjugated anti-rat IgG (lanes 1 and 3) or anti-mouse IgM (lanes 2 and 4) and NBT/BCIP substrates. The immunoprecipitating antibody (*IP aby*) is noted at the top of the figure and the immunoblotting antibody (*blot aby*) is noted at the bottom. Markers indicating the 116- and 97-kD molecular mass standards are shown at the right.

The extract was immunoprecipitated with Sepharose-conjugated anti- β -catenin (10C4; lanes 3 and 4) or control beads with coupled mouse IgG (lanes 1 and 2), as described in Materials and Methods. The precipitates were washed with stringent buffer, eluted with Laemmli buffer, and resolved by SDS-PAGE using a 7% acrylamide gel. The proteins were transferred electrophoretically to nitrocellulose and probed with anti-E-cadherin (E-9; lanes 1 and 3) or anti- α -actinin (BM-75.2; lanes 2 and 4) followed by alkaline phosphatase-conjugated anti-rat IgG (lanes 1 and 3) or anti-mouse IgM (lanes 2 and 4) and NBT/BCIP substrates. The immunoprecipitating antibody (*IP aby*) is noted at the top of the figure and the immunoblotting antibody (*blot aby*) is noted at the bottom. Markers indicating the 116- and 97-kD molecular mass standards are shown at the right.

α -catenin, but not actin. We therefore propose that the cadherin/catenin complex is linked to the actin cytoskeleton via a direct interaction between α -actinin and α -catenin.

Previously, it was proposed that vinculin links the cadherin/catenin adhesion complex to the actin cytoskeleton. In part, this idea was based on vinculin's extensive colocalization with E- and N-cadherin in epithelial cells and the myocardium, respectively. Here we show that vinculin does not colocalize extensively with the N-cadherin/catenin complex in fibroblasts. In contrast, α -actinin colocalizes extensively with the complex in both fibroblasts and epithelial cells. These observations stimulated us to consider the possibility that α -actinin interacts directly with the cadherin/catenin complex and serves to link it to the actin cytoskeleton.

In support of this idea, a series of experiments presented here shows that α -actinin coimmunoprecipitates specifically with catenins and the N-cadherin/catenin complex. Several lines of evidence support the specificity of the interaction. α -actinin was detected in immunoprecipitates captured using several different mAbs, including those to N-cadherin, α -catenin, and β -catenin. In contrast, it was not detected in nonimmune control precipitates; nor was it detected in immunoprecipitates missing either α -catenin or the cadherin.

Together, three lines of evidence imply that α -actinin interacts with α -catenin in cadherin/catenin complexes. First, α -actinin was present in the anti-N-cadherin immunoprecipitate, which contains only cadherin-bound catenins, suggesting it binds to the complex either via the cytoplasmic domain of the N-cadherin or, more likely, via either α - or β -catenin. The fact that α -actinin failed to coimmunoprecipitate with E-cadherin/ β -catenin complexes from PC9 cells missing α -catenin argues that α -actinin interacts with cadherin/catenin complex via α -catenin. This does not necessarily rule out a possible α -catenin-dependent interaction with the cadherin cytoplasmic domain, however. α -actinin did not associate with α -catenin when it was im-

muno-precipitated from the soluble catenins. This suggests that the presence of the cadherin is required for α -actinin to associate with α -catenin. We do not currently understand why this is the case. Perhaps the cadherin enhances or stabilizes α -actinin's interaction with α -catenin in the complex via conformational changes involving direct or indirect interactions. Alternatively, a posttranslational event occurring at the plasma membrane, where the cadherin is located and binds the catenins, may act to enhance or stabilize the interaction of α -actinin with α -catenin.

Recently, Jon Morrow and his collaborators have presented evidence that bacterially expressed α -catenin binds and bundles actin (Rimm, D. L., E. R. Koslov, P. Kebriaei, and J. S. Morrow. 1994. Alpha-catenin is an actin binding and bundling protein. *Mol. Biol. Cell.* 5 (Suppl.):5a). This raises the possibility that α -actinin can associate with the cadherin/catenin complex via actin. However, we detected a strong α -actinin signal in immunoprecipitates that contained only a trace actin signal, indicating that α -actinin can associate with the cadherin/catenin complex in the absence of actin. Our data and those of the Morrow lab are not necessarily inconsistent, however. It is feasible that both actin and α -actinin interact directly with α -catenin. This might not be surprising considering α -catenin shares homology with vinculin (Herrenknecht et al., 1991; Nagafuchi et al., 1991), which is known to bind both α -actinin and actin (Belkin and Koteliensky, 1987; Wachsstock et al., 1987; McGregor et al., 1994; Menkel et al., 1994). Moreover, Nagafuchi et al. (1994) recently reported that α -catenin interacts with the cytoskeleton by two different mechanisms, one via the carboxy-half, which results in full cadherin adhesive activity, and the other via the amino-half, which does not support full cadherin activity.

Although it is not possible to confidently determine stoichiometry from our experiments, the immunoblots suggest that the ratio of cadherin/catenin complex to α -actinin present in cell extracts is less than 1:1. There are at least two possible reasons for this. First, it is possible that not all of the complex is bound to α -actinin. In addition, the α -actinin may disassociate from the complex during the extraction/precipitation procedures. In vivo, the interaction between α -actinin and the cadherin/catenin complex might be stabilized, perhaps via posttranslational modifications or the presence of additional proteins.

Vinculin colocalizes extensively with the cadherin/catenin complex in some cells (e.g., epithelial cells) but not others (e.g., fibroblasts). This suggests that two types of cadherin-containing cell-cell adherens junctions may exist in cells, those with, and those without, vinculin. Although we did not detect vinculin in any of our immunoprecipitates, even those from epithelial cells, it is possible that our extraction/immunoprecipitation conditions either disrupted vinculin's interaction with the complex or failed to solubilize vinculin-containing complexes. Through its ability to bind both actin and α -actinin, vinculin may stabilize the adherens junction in cells that form extensive and stable cell-cell contacts. How the association of vinculin with the cell-cell adhesion complex is regulated in different cell types is a question for future study.

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