α-Catenin Binds Directly to Spectrin and Facilitates Spectrin-Membrane Assembly in Vivo*

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The anchorage of spectrin to biological membranes is mediated by protein and phosphoinositol phospholipid interactions. In epithelial cells, a nascent spectrin skeleton assembles in regions of cadherin-mediated cell-cell contact, and conversely, cytoskeletal assembly is required to complete the cell-adhesion process. The molecular interactions guiding these processes remain incompletely understood. We have examined the interaction of spectrin with α-catenin, a component of the adherens complex. Spectrin (αIIβII) and α-catenin co-precipitate from extracts of confluent Madin-Darby canine kidney, HT29, and Clone A cells and from solutions of purified spectrin and α-catenin in vitro. By surface plasmon resonance and in vitro binding assays, we find that α-catenin binds αIIβII spectrin with an apparent $K_d$ of 10–100 nM. By gel-overlay assay, α-catenin binds recombinant βI-spectrin peptides that include the first 313 residues of spectrin but not to peptides that lack this region. Similarly, the binding activity of α-catenin is fully accounted for in recombinant peptides encompassing the NH2-terminal 228 amino acid region of α-catenin. An in vivo role for the interaction of spectrin with α-catenin is suggested by the impaired membrane assembly of spectrin and its enhanced detergent solubility in Clone A cells that harbor a defective α-catenin. Transfection of these cells with wild-type α-catenin reestablishes α-catenin at the plasma membrane and coincidentally recruits spectrin to the membrane. We propose that ankyrin-independent interactions of modest affinity between α-catenin and the amino-terminal domain of β-spectrin augment the interaction between α-catenin and actin, and together they provide a polyvalent link directing the topographic assembly of a nascent spectrin-actin skeleton to membrane regions enriched in E-cadherin.

The spectrin-actin cytoskeleton contributes to membrane structure and provides molecular linkages between organized membrane domains and the filamentous cytoskeleton (reviewed in Refs. 1–4). Its assembly on any given membrane is guided by interactions with membrane proteins and phosphoinositol phospholipids (5–8). Best understood are linkages involving the adapter protein ankyrin. Ankyrin binds spectrin with high affinity (~10–100 nM), linking spectrin to a variety of transmembrane proteins including ion channels or pumps, such as the anion exchanger AE1, the voltage-gated Na⁺ channel, Na⁺/K⁺-ATPase, H⁺/K⁺-ATPase (9–13), and cell adhesion molecules of the Ig superfamily such as neurolgin, neurofascin, or NrCAM (7, 14–16). Other recognized adapter proteins linking spectrin to the membrane include adducin and members of the protein 4.1 superfamily, 4.1R, 4.1B, and 4.1G (reviewed in Refs. 4 and 17). Direct interactions of spectrin with the membrane are mediated by at least three distinct regions of β-spectrin, termed membrane association domains 1, 2, and 3 (MAD1,1 MAD2, and MAD3). MAD1 is located in repeat unit 1 of both βI and βII spectrin (5) and contains a constitutive targeting signal that together with sequences in region 1 of spectrin can direct spectrin to either the Golgi or plasma membrane of MDCK cells (18,2,3 MAD2, found in the COOH-terminal pleckstrin homology domain of all spectrins except βI2 (4), binds both a protein ligand (5, 6) as well as phosphatidylinositol-4,5-P2 phospholipid (20, 21). MAD2 may participate in mediating G-protein control on the assembly process (21–23). MAD3 involves a region within β-spectrin repeats 3–9 (6), its function remains uncharacterized, although it has been proposed to interact with a membrane calmodulin-binding protein (24). Other modes of attachment and other adapter proteins almost certainly exist; their identification remains central to a complete understanding of spectrin assembly and function.

In epithelial cells, assembly of the nascent cortical spectrin skeleton occurs at zones of cell-cell contact, regions where there is productive Ca²⁺-mediated homophilic adhesion between surface E-cadherin molecules (8, 25, 26). Associated with E-cadherin is a group of cytoplasmic proteins that include α- and β-catenin (or γ-catenin) and p120 (for review see Refs. 25 and 27). β-Catenin (or in some cells γ-catenin) directly binds the cytoplasmic domain of E-cadherin; α-catenin joins the membrane complex via a direct association with β-catenin or γ-catenin (28). α-Catenin binds and bundles F-actin, an activity that presumably facilitates the attachment of actin filaments to the adhesion complex (29). α-Catenin also binds α-actinin, a dis...
tant member of the spectrin gene superfamily; this interaction may facilitate the docking of actin at the adhesion complex (30).

The catenins, and their assembly with the cortical cytoskeleton, are closely linked to the regulation of cadherin function (25, 31, 32). Spectrin assemblies with the adhesion complex soon after productive cell-cell contact is established (26). The interactions guiding this process remain undefined. We now report a direct interaction of spectrin with α-catenin, and we demonstrate that α-catenin is required for spectrin assembly at the plasma membrane in Clone A cells, a human intestinal cell line. These studies thereby identify α-catenin as a novel adapter protein mediating spectrin-membrane association, suggest that this association is necessary for maturation of at least some types of cadherin-mediated junctions, and provide insight into the molecular mechanisms by which spectrin participates in the establishment of specialized membrane domains in polarized cells.

EXPERIMENTAL PROCEDURES

Construction of Expression Plasmids—Molecular biological procedures followed standard protocols (33). Recombinant peptides were prepared from coli as bacterial overexpressed α-catenin systems (Amersham Pharmacia Biotech, also see Refs. 29 and 34). Inserts of cDNA encoding selected regions of α-catenin were isolated from a human colon cDNA library (GenBank™ accession number L23805, see Ref. 35). The desired region of the cDNA was digested with appropriate restriction endonucleases; the inserts were purified from agarose gels with the Qiaex gel extraction kit (Qiagen) and subcloned into pGEX-2T. The alignment with the α-catenin sequence is shown in Fig. 2 and as used previously (25, 31, 32). Spectrin assembles with the adhesion complex (29) and the binding to GST fusion proteins was completely precipitated. Binding to GST alone or to protein-A beads in the absence of fusion protein was used as controls for nonspecific binding and were subtracted from all results to obtain specific binding data.

Overlap Binding—GST was cleaved from recombinant α-catenin by thrombin digestion (28). GST-spectrin peptides (1.0, 0.5, and 0.1 μg) were analyzed by SDS-PAGE on 7.5% gels and transferred to PVDF membranes. Membranes were blocked for 1 h in 5% BSA in Tris-buffered saline (TBS); pH 7.5, and overlayed for 1 h with 0.5 mg/ml recombinant α-catenin. After five brief rinses with TBS, a 1:1000 dilution of monoclonal antibody 3H4 was overlayed for 1 h at RT. After five additional washes, goat anti-mouse antibody at a 1:10,000 tagged with horseradish peroxidase was used with enhanced chemiluminescence (Amersham Pharmacia Biotech). Bound α-catenin was visualized with ECL (Amersham Pharmacia Biotech).

Surface Plasmon Resonance—Binding detection by surface plasmon resonance was implemented using a Biacore™ 1000 or 2000 instrument (Biacore AB). This technique of detecting protein-protein interactions is fully described in several publications (e.g. see Refs. 42–44). Purified αIIβIII spectrin was immobilized onto a carboxymethylated dextran gold surface of the Biacore™ chip in 100 mM acetate buffer, pH 4.5, 50 mM Na2HPO4, and 0.2 mM N-ethyl-N’-dimethylaminopropyl) carbodiimide hydrochloride. Several chip surfaces were prepared, ranging from 350 to 1500 RU’s of bound spectrin. Purified recombinant human α-catenin or expressed α-catenin subdomains were injected onto the spectrin surface, and the binding was measured as an increase in the resonance units (RU). The kinetic constants, kD and kcat, for the binding of α-catenin to spectrin were determined as described (40) from a plot of dRU/dt versus R (nM) versus concentration. The slope of these plots are equal to kD and the abcissa intercept equal to kcat. The equilibrium dissociation value is determined from the equation Kd= kcat/kD. Alternatively, the sensograms were fit using the nonlinear fitting algorithms for multisite binding provided by Biacore AB. Residuals were calculated against systems derived from the fitting algorithms as a measure of the appropriateness of the binding model.

The binding surfaces were regenerated between determinations with 10 mM HCl or 10 mM NaOH. Control studies established the stability of the binding surfaces over the course of these experiments. Binding buffer conditions were 10 mM HEPES, 50 mM NaCl, 1 mM dithiothreitol, 0.5 mM EDTA, 1 mM Na2SO4, pH 6.8.

Transfection Procedure—Clone A cells (5 × 105) were plated in 60-mm Petri dishes, incubated overnight, and transfected using the LipofectAMINE reagent (Life Technologies, Inc.) with 3–6 μg of the plasmid pcDNA3 (Invitrogen) carrying full-length human α1(E)-catenin (pcDNA-α1-catenin) (35). The manufacturer’s transfection protocol was followed without modification. When stable lines were desired, neomycin-resistant clones were isolated by selective growth in medium containing 0.6 mg/ml of G418 (Life Technologies, Inc.). Subclones were identified on the basis of their assumption of a more sheet-like morphology, a phenotypic change in Clone A cells characteristic of full-length wild-type α-catenin expression (46). Alternatively, since stable lines often proved unstable, transiently expressing cells were used for most experiments. The expression of α-catenin was verified by immunofluorescence and Western blotting.

Immunofluorescence—Cells were plated onto glass chamber slides and grown to confluence. Cells were washed five times in TBS, pH 8.0, and fixed in methanol for 20 min at 4 °C. After fixation, cells were washed again and blocked with 0.3% BSA/TBS for 1 h at RT. After blocking, cells were washed and incubated with various primary antibodies (diluted in 0.5% BSA/TBS) for 1 h at RT. Antibodies were used at the following concentrations: monoclonal antibodies α-catenin (6A5) and 7A11 were used as undiluted culture supernatants; polyclonal antibody (RAF) to αII spectrin was used at 1:500 dilution. After incubation with primary antibodies, cells were washed and incubated with CY3- or CY2-conjugated secondary antibodies (Jackson Immunoresearch) diluted 1:500 in 0.3% BSA/TBS for 1 h at RT. Cells were finally washed, mounted, and viewed by phase and epifluorescence using an Olympus
intercellular cadherin-mediated junctions. When these cells become more confluent and establish effective cell-cell contact, the distribution of α-catenin (A and D) or αIIβII spectrin (B and E) were evaluated by indirect immunofluorescence in sparse (A–C) or partially confluent (D–F) cultures of HT29 cells. Note that whereas sparse cultures display high cytoplasmic concentrations of both α-catenin and spectrin, these proteins assemble together along zones of productive cell-cell contact, with reduction of the cytoplasmic concentrations as cells grow to confluence. This is most evident in the merged images (C and F). Bar = 10 μm.

**RESULTS**

**αIIβII Spectrin and α-Catenin Associate in Vivo**—Several studies have established the coincidence at the light microscopic level of plasma membrane-associated spectrin and the cadherin-catenin adhesion complex along zones of cell-cell contact. A comparison of the pattern of spectrin staining in Clone A cells and HT29 cells (a closely related colonic epithelial cell line) suggests that α-catenin may mediate the linkage of spectrin to the adhesion complex. In poorly confluent HT29 cells, spectrin is largely cytoplasmic, as is α-catenin (Fig. 1, A–C). When these cells become more confluent and establish effective intercellular cadherin-mediated junctions, α-catenin is recruited to zones of cell-cell contact (Fig. 1D). Coincident with this recruitment of α-catenin, there is recruitment of spectrin from cytoplasmic to membrane pools, in a distribution indistinguishable from α-catenin (Fig. 1, D and E; Fig. 2). Conversely, in Clone A cells, an internal deletion of exons 4 and 5 in the transcribed cDNA of α-catenin generates a shortened and mutated α-catenin transcript (Fig. 3, also see Refs. 46 and 47). Clone A α-catenin is unstable and fails to associate with the cadherin-based adhesion complex (39, 46), even though its ability to associate with β-catenin and actin appear qualitatively unimpaired (46). As a result, intercellular adhesion in Clone A cells is impaired. Also impaired is the assembly of spectrin to the plasma membrane. This is evident both by its...
GST removed). Their M did not change its affinity for spectrin (data summarized in Table I). Cells, with the internal deletion of sequences encoded by exons 4 and 5, were used in binding assays either as a GST fusion peptide or as the recombinant protein alone after removal of the GST by thrombin. Bovine αIβII spectrin was immobilized on the Biacore \( ^\text{TM} \) sensor chip surface, and the changes in resonance units were monitored for different concentrations of α-catenin (Fig. 3B) or for different α-catenin subdomains (Fig. 3C). Sensograms (not shown) were also obtained for different surface loadings of spectrin, to evaluate artifacts arising from limitations of mass transport to the sensor surface. In each analysis, the earliest portions of the association and dissociation phases were analyzed. These are the regions where mass transport (during association) and rebinding (during dissociation) do not dominate the sensogram. Each sensogram was fit to several different binding models, as provided in the Biacore software package, including simple one-to-one Langmuir binding isotherms and two-exponential models. All models generated reasonable fits, with apparent values of \( k_{\text{on}} \), \( k_{\text{off}} \), and \( K_D \) within a factor of \( \sim 3 \) of each other for a given peptide. However, no model generated fits with fully random residuals, indicating that the binding of α-catenin to immobilized spectrin, although real and of high affinity, is complex and does not conform to any simple binding model. A summary of the apparent kinetic values and derived apparent \( K_D \) values for α-catenin binding to αIβII spectrin is presented in Table I. In this analysis, wild-type α-catenin bound spectrin with an apparent \( K_D \) of 19–80 nM, GST-α-catenin bound with an apparent \( K_D \) of 19–24 nM, and the mutant Clone A α-catenin, devoid of GST, bound with an apparent \( K_D \) of 15–25 nM. The differences in apparent binding affinity between the GST-α-catenin versus peptides without GST presumably reflects the propensity of GST to induce homodimerization. Oligomers of α-catenin generated by this mechanism would bind with enhanced affinity, a phenomenon that has now been well documented (19). A surprise is the apparently greater affinity of GST-free Clone A α-catenin for spectrin versus wild-type α-catenin. The genesis of this effect is unknown and was not further studied, although such a finding does suggest the possibility that the Clone A mutation might affect the oligomerization pathway of native α-catenin (28).

To identify the site in α-catenin that interacts with spectrin, additional recombinant peptides derived from the NH\( _2 \) and COOH termini of α-catenin (Fig. 3A) were prepared and assayed qualitatively for their ability to bind directly to αIβII spectrin using surface plasmon resonance (Fig. 3C). As before, full-length α-catenin (peptide 907) bound avidly. Peptide N576, representing the NH\( _2 \)-terminal half of α-catenin, bound in a similar way, achieving approximately half of the RUs of peptide 907. A peptide representing the NH\( _2 \)-terminal 228 residues of α-catenin also bound. Since the Biacore instrument measures a mass change at the sensor surface, the decrease in RU values seen with the various NH\( _2 \)-terminal peptides are in proportion to their relative molecular weights and indicate that all of these peptides, loaded at equal concentrations, are saturating the spectrin surface to the same extent. Conversely, the C447 peptide, encompassing the COOH-terminal half of α-catenin, did not bind to spectrin any better than persistent cytoplasmic intracellular distribution even in confluent monolayers of Clone A cells (Fig. 2A), as well as by its reduced resistance to extraction with Triton X-100 (compared with HT29 cells, an intestinal line with normal α-catenin, Fig. 2B). α-Catenin can also be directly demonstrated in immunoprecipitates of αIβII spectrin solubilized from confluent MDCK, HT29, and Clone A cells (Fig. 2C); MDCK cells are an epithelial line with well documented spectrin association at cadherin-based junctions (e.g. see Ref. 8). Collectively, these \( \text{in vivo} \) observations indicate a tight and possibly direct link-

**FIG. 3.** βII spectrin binds directly to the NH\( _2 \)-terminal 228 amino acids (aa) of α-catenin. A. Schematic representation of the structure of α-catenin, the mutation found in Clone A cells (shaded box (46)), and the recombinant α-catenin peptides used in this study. On the right is shown an SDS-PAGE analysis, Coomassie Blue-stained, of the purified αIβII spectrin (S) and each of the α-catenin peptides (with GST removed). Their \( M_\text{r} \) 1000 is shown. B, surface plasmon resonance analysis of the interaction of GST-α-catenin with immobilized αIβII spectrin. A representative analysis is shown. Binding was evaluated on sensor chips containing three different concentrations of spectrin (see “Experimental Procedures”). Each curve (from bottom to top) represents the sensogram trace of the binding of 0.15, 0.30, 0.60, 1.2, and 2.4 μM recombinant GST-α-catenin, respectively. Similar experiments carried out with different levels of immobilized spectrin, and with recombinant α-catenin in which the GST moiety had been removed by thrombin treatment, gave comparable results. Interestingly, analysis of recombinant α-catenin representing the form found in Clone A cells, with the internal deletion of sequences encoded by exons 4 and 5, did not change its affinity for spectrin (data summarized in Table I). C, comparison sensogram of the binding of various GST-α-catenin peptides at 0.5 μM to αIβII spectrin. The α-catenin peptides are as indicated. Note that only those peptides containing the NH\( _2 \)-terminal 228 residues demonstrate appreciable binding and that the level of binding achieved in the sensogram at saturation (for active peptides) is roughly proportional to the mass of each active peptide. D, solution binding of αIβII spectrin to α-catenin peptides. Increasing concentrations of \( ^{35} \)S-labeled αIβII spectrin were incubated with α-catenin peptides N576 (■) or C447 (○). The binding of spectrin to GST alone or to the protein A beads in the absence of fusion protein was taken as a measure of nonspecific binding.
Spectrin Binding to α-Catenin

The values presented represent only an apparent $K_D$, since in neither case (simple bimolecular Langmuir binding or two-exponential fit) were the fitting residuals completely random. Several mechanisms may contribute to this complexity, including surface microheterogeneity, limitations in mass transport to and from the binding surface, and complex conformational dependent binding mechanisms inherent to the proteins themselves. Available data are insufficient to discriminate between these possibilities. The data do demonstrate unequivocal high affinity binding of α-catenin to spectrin.

### Table I

| Peptide          | Apparent $K_D$ $^{a}$ | $k_a$ $^{a}$ | $k_d$ $^{a}$ | Linear fit $^{b}$ |
|------------------|-----------------------|--------------|--------------|-------------------|
| α-Catenin        | 68.0 ± 4.9            | 5.5 ± 0.1E4  | 3.7 ± 0.2E-3 | 76.0 ± 4.5        |
| Clone A          | 15.3 ± 3.7            | 5.6 ± 0.03E4 | 0.9 ± 0.2E-3 | 24.5 ± 6.2        |
| GST-α-catenin    | 19.0 ± 1.6            | 14.0 ± 0.1E4 | 2.7 ± 0.2E-3 | 24.0 ± 2.0        |

| Peptide          | Apparent $K_D$ $^{b}$ | $k_a$ $^{b}$ |
|------------------|-----------------------|--------------|
| α-Catenin        | 6.2 ± 3.5             | 0.03E4       |
| Clone A          | 4.5 ± 0.2             | 0.1E4        |
| GST-α-catenin    | 11.0 ± 0.1E4          | 0.2E-3       |

$^{a}$ Values ± S.E., calculated using Biaevaluation version 3.0, model 1:1 Langmuir binding.
$^{b}$ $K_D$ calculated using $k_a$ from the exponential fit.

**Fig. 4. The first 313 residues of βII spectrin effect α-catenin binding.** A, summary of the recombinant βII spectrin peptides used in relation to the overall functional domain structure of βII spectrin. The major known ligand-binding regions in βII spectrin are depicted, as are the codons at the beginning and end of each of the recombinant peptides. ABD, actin-binding domain; MAD, membrane association domain α; ANK, ankyrin binding domain; PH, pleckstrin homology domain. B, solid phase blot assay of recombinant α-catenin to βII spectrin peptides. Left, Coomassie Blue-stained SDS-PAGE analysis of the GST-spectrin peptides, with their molecular weights. Center, PVDF transfer of spectrin recombinant peptides, overlaid with α-catenin and developed with the 3H4 antibody to α-catenin. Note the strong binding of α-catenin to βII$_{L_C}$, βII$_{N_A}$, and βII$_{C_1}$ and the absence of binding to the other spectrin peptides or to GST alone. Data from peptides prepared as a fusion with GST or as the peptide alone after removal of the GST are shown. Right, PVDF transfer of βII$_{C}$, βII$_{N_1}$, or GST alone at three loadings (1.0, 0.5, or 0.1 μg) overlaid with α-catenin (GST-free). Note the strong binding to βII$_{N_1}$, with no detectable binding to βII$_{C}$ or GST at any concentration.

Finally, it was of interest to determine whether α-catenin would also bind αIIβII spectrin in vitro in solution (versus immobilized spectrin on the Biacore sensor surface). Increasing concentrations of purified 125I-labeled αIIβII spectrin were mixed with either of the recombinant GST fusion proteins N576 or C447. Antibodies to GST were used to co-purify GST-catenin along with bound 125I-labeled spectrin, which was quantified by $\gamma$-counting (Fig. 3D). Nonlinear regression analysis of the binding isotherm (fitted line) revealed $K_D$ values of 164 ± 86 (2 S.D.) nM for spectrin binding to GST-N576, with an estimated $K_{max}$ of 0.50 ± 0.12 (2 S.D.) mol of spectrin dimer bound per mol of GST-N576. In these assays, there was no binding of spectrin to GST-C447 or GST alone. The $K_D$ determined from this assay agreed reasonably well with those from the Biacore studies, especially considering the differences in technique. Collectively, they demonstrate a strong and direct interaction between αIIβII spectrin and the NH$_2$-terminal 228 residues of α-catenin. Consistent with this binding locus, no differences in spectrin binding by Clone A α-catenin were detected. Clone A α-catenin deletes residues 197–354 of the native protein, suggesting that the actual interaction site in α-catenin for spectrin is proximal to residue 197.

**α-Catenin Binds to the First 313 Residues of βII Spectrin**—The site to which α-catenin binds in βII spectrin was identified by gel-overlay assay (Fig. 4). Recombinant GST fusion peptides representing all regions of human βII spectrin were transferred to PVDF membranes and overlaid with α-catenin (from which GST had been removed) (Fig. 4). Of the peptides examined, only those (βII$_{N_2}$, βII$_{N_4}$, and βII$_{N_4}$) that included the NH$_2$-terminal region of βII spectrin bound α-catenin (Fig. 4B, center). To assess further the relative affinities of α-catenin for this region of βII spectrin, overlay experiments were designed using a range of peptide concentrations (Fig. 4B, right). Regardless of concentration, α-catenin did not bind to GST alone or to βII$_{N_4}$. Conversely, strong binding was detected at every
concentration of the βII1,1 peptide. This active peptide, the smallest one examined in these experiments, spans residues 1–313 of βII spectrin and places the α-catenin-binding site within this region.

α-Catenin Facilitates Spectrin Membrane Assembly in Vivo—

Clone A cells are defective in cell-cell adhesion and harbor an internal deletion in the expressed α-catenin (39, 46). This mutation leads to the loss of α-catenin associated with the plasma membrane, reduced cell-cell adhesion, and coincidentally, reduced assembly of αIIβII spectrin at the membrane (Figs. 2 and 5). These cells do, however, form epithelial-like sheets at confluence (albeit with altered morphology and highly refractile membranes) and display surface E-cadherin and β-catenin staining (46). To test whether the failure of spectrin assembly in these cells was due to the defect in α-catenin, Clone A cells were transiently transfected with wild-type α-catenin, and the assembly of α-catenin and αIIβII spectrin at the membrane was monitored (Fig. 5). The transfected wild-type α-catenin was fully competent for assembly with the cadherin adhesion complex at the plasma membrane (Figs. 5, A and C), and coincident with its appearance, αIIβII spectrin was restored to its plasma membrane location (Fig. 5, B and D). Thus, wild-type α-catenin is fully competent to rescue the impaired membrane assembly of spectrin in Clone A cells.

DISCUSSION

These findings establish that α-catenin can bind directly to spectrin, that these proteins are associated in cultured epithelial cells in vivo, and that α-catenin facilitates the plasma membrane assembly of spectrin in regions of direct cell-cell contact. These conclusions are supported by several lines of evidence as follows. (i) In vitro coprecipitation, surface plasmon resonance, and gel overlay assays detect a direct interaction of moderate to high affinity between the NH2-terminal domain of α-catenin and the first 313 residues of βII spectrin. (ii) Spectrin and α-catenin co-localize and co-precipitate in confluent monolayers of Clone A, HT29, and MDCK cells. (iii) Spectrin and α-catenin do not assemble into a detergent-insoluble matrix at the plasma membrane in clone A cells, a defect restored by transfection of wild-type α-catenin. Collectively, these findings suggest that in addition to its other roles, α-catenin acts as a novel adapter protein directing the assembly of a nascent cortical spectrin membrane skeleton to zones of productive cell-cell adhesion. It is also likely that the binding of spectrin to the adhesion complex stabilizes the complex itself and facilitates adhesion by linking adjacent adhesion complexes into macro-molecular membrane mosaics centered at cadherin-based junctions. In this respect, the interaction of spectrin with the adhesion complex is but a specific example of the more general role of spectrin as an organizer of linked membrane mosaics (2, 4).

The demonstration of a direct interaction between α-catenin and spectrin is reminiscent of the binding of α-catenin to actinin (30). A member of the spectrin gene superfamily, α-catenin shares the repeat structure of spectrin and binds F-actin. However, unlike for spectrin, a sequence in the two central α-catenin repeat units appears to intersect with a region in α-catenin that is downstream of the spectrin-binding site identified here. This result is a bit surprising given the similarity of α-catenin to spectrin and suggests that spectrin and α-catenin, despite their similarities, play distinct roles in the physiology of the adhesion complex. The presence of distinct binding sites for both α-catenin and spectrin (and actin, Ref. 29) in α-catenin, as well as independent binding sites for actin in both spectrin and α-catenin, suggests that these molecules can bind simultaneously and independently (although this premise has not been formally examined). Thus, it is likely that a cooperative and redundant interaction of α-catenin with spectrin, F-actin, and α-catenin guides the assembly of a spectrin-actin skeleton to regions of cell-cell contact. The findings in Clone A cells lend support to this notion. Although α-catenin from Clone A cells binds spectrin normally (as it does F-actin and β-catenin (46)), the deletion in this catenin (residues 197–354) overlaps a region previously demonstrated to bind α-catenin (residues 325–394 (30)). Perhaps a loss of α-catenin binding leads to an impairment of actin assembly at the membrane, with consequential impairment of spectrin-actin assembly in zones of cell-cell contact.

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Fig. 5. Wild-type α-catenin restores spectrin assembly at the membrane in Clone A cells. Clone A cells were transiently transfected with wild-type α-catenin and stained with the monoclonal antibody 7A11, which only recognizes wild-type α-catenin (46) (A and C) or with RAF-A, an antibody to αIIβII spectrin (B and D). Note the cluster of transfected cells that express α-catenin at the membrane and assume a more epithelial morphology. As shown in higher power in the bottom panels (C and D), the spectrin in these cells shifts from a largely cytoplasmic distribution in the untransfected Clone A cells (arrows, D), to a predominantly plasma membrane association (arrowheads, D).
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