Genetic and biochemical dissection of protein linkages in the cadherin–catenin complex

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ABSTRACT The cadherin–catenin complex is important for mediating homotypic, calcium-dependent cell–cell interactions in diverse tissue types. Although proteins of this complex have been identified, little is known about their interactions. Using a genetic assay in yeast and an in vitro protein-binding assay, we demonstrate that β-catenin is the linker protein between E-cadherin and α-catenin and that E-cadherin does not bind directly to α-catenin. We show that a 25-amino acid sequence in the cytoplasmic domain of E-cadherin and the amino-terminal domain of α-catenin are independent binding sites for β-catenin. In addition to β-catenin and plakoglobin, another member of the armadillo family, p120 binds to E-cadherin. However, unlike β-catenin, p120 does not bind α-catenin in vitro, although a complex of p120 and endogenous α-catenin could be immunoprecipitated from cell extracts. In vitro protein-binding assays using recombinant E-cadherin cytoplasmic domain and α-catenin revealed two cadherin pools in cell lysates: an ~1000- to ~2000-kDa complex bound to E-cadherin and an ~220-kDa pool that did not contain E-cadherin. Only β-catenin in the ~220-kDa pool bound exogenous E-cadherin. Delineation of these molecular linkages and the demonstration of separate pools of catenins in different cell lines provide a foundation for examining regulatory mechanisms involved in the assembly and function of the cadherin–catenin complex.

The cadherin superfamily comprises glycoproteins responsible for calcium-dependent, homotypic cell interactions (1). E-cadherin is generally expressed in epithelial tissues and has been shown to regulate cell–cell adhesion (2), cell migration (3), morphogenesis (4), and the establishment of membrane polarity (5).

Homotypic interactions between extracellular domains of cadherins are necessary but not sufficient for cell–cell adhesion (2). Linkage of the cadherin cytoplasmic domain to three cytosolic proteins, named α-catenin, β-catenin, and plakoglobin (γ-catenin), is required (6–8). Although α-catenin, β-catenin, and plakoglobin can be communoprecipitated in a complex with E-cadherin (1, 7), the binding order of protein–protein interactions has not been resolved. Insight into this problem is important for understanding functions of the cadherin–catenin complex and the regulation of cadherin–catenin complex assembly. Here, we define the binding order of protein–protein interactions in the cadherin–catenin complex using genetic and biochemical approaches.

MATERIALS AND METHODS

Strain and Microbiological Techniques. All cloning procedures and bacterial transformation were performed by standard procedures outlined by Sambrook et al. (9). Yeast strain Y190 (MATa gal4 gal80 his3-200 trp1-901 ade2-101 ura3-52

leu2-3,112 his2-801 URA3::GAL→lacZ LYS2::GAL→HIS3 was used in the two-hybrid analysis and was provided by Stephen J. Elledge (Baylor College of Medicine, Houston). Yeast transformations were performed by the lithium acetate/polyethylene glycol method (10). A 499-bp DNA fragment corresponding to the coding sequence of the cytoplasmic domain of canine E-cadherin (E-cad(CD)) was generated by PCR with synthetic oligonucleotides 5’-GGATCCGAGAAGGTTGTGAAGAGCC-3’ and 5’-GGAGACGGTGCACTATCTTCTATTGT-TCTCAAGTCCTCATG-3’. This fragment was cloned into the BamHI and Sal I sites of pAS1CYH2 (provided by Stephen J. Elledge) to generate a GAL4 DNA-binding domain (GAL4ad)-E-cadCD fusion protein. The same PCR-amplified fragment was cloned into the Sal I and Xho I sites of pACT2 (provided by Stephen J. Elledge) to generate a GAL4 DNA transcriptional-activation domain (GAL4ad)-E-cadCD fusion protein; a similar construct [GAL4ad-E-cad(−25)] but with a 25-amino acid deletion from amino acids 815 to 839 E-cadCD was also made by using the construct pUM∆C25 (11). Full-length mouse β-catenin coding sequence, provided by Rolf Kemler, was cloned into Nde I and Xho I sites of pACT2 to generate a GAL4ad-β-catenin fusion protein (GAL4ad-β-cat). Full-length mouse α-catenin coding sequence, provided by Rolf Kemler, was fused in the correct open reading frame with pAS1CYH2 and pACT2 vectors to generate plasmids expressing GAL4ad-α-catenin and GAL4ad-α-catenin fusion proteins, respectively. In addition, α-catenin coding sequence corresponding to amino acids 1–606 was cloned in frame into GAL4ad vector to generate GAL4ad-α-catenin(N) fusion protein, and the coding sequence corresponding to the C-terminal 300 amino acids of α-catenin was fused in frame to generate GAL4ad-α-catenin(C) fusion protein. All plasmid constructs were confirmed by restriction analysis and DNA nucleotide sequencing. The glutathione S-transferase (GST)-E-cadCD fusion protein was previously constructed and characterized (12). Recombinant α-catenin was generated by cloning full-length α-catenin cDNA into the histidine-tag expression vector, pQE60 (Diagen, Chatsworth, CA). The resulting C-terminally tagged α-catenin was purified according to the manufacturer’s instructions (Diagen).

Two-Hybrid Analysis. Yeast strain Y190 was transformed with 1 µg of each transforming DNA. Transformants were selected for growth on SC medium (Bio 101) lacking tryptophan and leucine (SC–Trp–Leu) and then was restreaked

Abbreviations: GST, glutathione S-transferase; X-Gal, 5-bromo-4-chloro-3-indolyl β-d-galactoside; SV40, simian virus 40; T antigen, tumor antigen; E-cadCD, E-cadherin cytoplasmic domain; E-cad(−25), E-cadherin with a 25-amino acid deletion; GAL4ad, yeast GAL4 DNA-binding domain; GAL4cd, GAL4 transcriptional-activation domain; FPLC, fast protein liquid chromatography.*To whom reprint requests should be addressed: Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Beckman Center, B121, Stanford, CA 94305-5426.

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onto SC–Trp–Leu–His containing 50 mM 3-aminotriazole (3-AT; Sigma) or 5-bromo-4-chloro-3-indoyl-β-d-thiogalactoside (X-Gal) indicator plates (10) to select for cotransformants that had reconstructed a complex of the GAL4bd-GAL4ad domains. To quantitate β-galactosidase activity, transformants were grown in liquid medium containing SC–Trp–Leu at 30°C until the OD600 reached 0.5–1.0; cells were harvested, lysed, and assayed with o-nitrophenyl-β-d-galactoside (Sigma) as the chromogenic substrate for β-galactosidase activity (10). β-Galactosidase activity was normalized to protein concentration (Pierce BCA Kit).

**Cell Lines, Media, and Antibodies.** Human colon carcinoma HCT116, human colon adenocarcinoma SW480, and Madin-Darby canine kidney (MDCK) cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% (vol/vol) fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO2 at 37°C; human lung carcinoma PC9 cells (8) were grown in 1:1 (vol/vol) DMEM/F-12 medium containing 10% FBS. Rabbit antisera specific for GST–E-cadCD fusion protein, α-catenin, β-catenin, and plakoglobin, respectively, have been described and characterized (12–14). Mouse monoclonal anti-p120 antibody was purchased from Sigma.

**Metabolic Labeling and Binding Assay.** Cells were metabolically labeled with [35S]methionine/cysteine (DuPont/NEN) for 4 hr. For GST–E-cadCD binding assays, ∼107 HCT116, SW480, or MDCK cells or ∼107 PC9 cells were extracted with MEB buffer (50 mM Tris, pH 7.5/100 mM NaCl/0.5% Nonidet P-40) containing inhibitors [0.1 mM Pefabloc (Boehringer Mannheim), 0.1 mM Na3VO4, 50 mM NaF, and 0.01 mg of each chymostatin, leupeptin, antipain, and pepstatin A per ml]. The extract was precleared with glutathione-conjugated agarose and then incubated with either GST or GST–E-cadCD fusion protein bound to glutathione-agarose beads. After washing with MEB buffer, proteins were eluted from either glutathione-agarose or nickel-conjugated Sepharose by boiling the beads in 2× SDS sample buffer [100 mM Tris chloride, pH 6.8/200 mM dithiothreitol/4% SDS/0.2% bromophenol blue/20% (vol/vol) glycerol] and then separated in SDS/7.5% PAGE gels. Proteins were transferred from gels to nitrocellulose and immunoblotted as described (15). Antibodies were detected by using 125I-labeled protein A or 125I-labeled goat anti-mouse IgG (Amersham), visualized by autoradiography, and quantified by using a phosphor imager (Fujix BAS 2000, Fuji). For in vitro binding assays with histidine-tagged α-catenin, 2.5 × 109 MDCK cells were lysed in 250 μl of MEB buffer, and the soluble fraction was precleared by using 20 μl of nickel-Sepharose bound to Escherichia coli extract at 4°C for 1 hr in the presence of 10 mM imidazole. The supernatant was incubated with 20 μl of nickel-Sepharose saturated with histidine-tagged α-catenin as described above. To remove unbound protein, the pellet was extensively washed with MEB buffer containing 10 mM imidazole. Proteins were eluted with 20 μl of 100 mM EDTA and were heated in 2× SDS sample buffer at 37°C for 10 min, separated in SDS/7.5% PAGE gels, and processed for immunoblotting as above.

**RESULTS AND DISCUSSION**

**Determining the Hierarchy of Molecular Interactions in the Cadherin–Catenin Complex by Using the Yeast Two-Hybrid System.** We analyzed interactions between proteins in the cadherin–catenin complex using the yeast two-hybrid system (16, 17). Fig. 1 shows the growth phenotypes of yeast Y190 transformants as SC–Trp–Leu without 3-AT and SC–Trp–Leu–His with 50 mM 3-AT. All transformants grew on SC–Trp–Leu plates, which select for nutrient markers encoded by the GAL4bd and GAL4ad plasmids. However, growth on SC–Trp–Leu–His with 50 mM 3-AT was restricted to cotransformants expressing E-cadherin–β-catenin, α-catenin–β-catenin, or p53-simian virus 40 (SV40) large tumor (T)-antigen (positive control). Growth of cotransformants in the latter medium is consistent with expression of corresponding fusion proteins and subsequent reconstitution of GAL4 transcription factor function. Cotransformants expressing α-catenin–E-cadherin did not grow, indicating that fusion proteins containing α-catenin and E-cadherin cytoplasmic domain did not interact. Control experiments were performed with Y190 transformed with individual plasmids that were used in cotransformation growth assays. The resulting transformants did not survive His− and 3-AT selection (data not shown), demonstrating that individual fusion proteins did not activate the his3 reporter gene.

![Fig. 1. Assay for interactions between E-cadCD and catenins by using the yeast two-hybrid system. Yeast cotransformants were grown on SC–Trp–Leu medium without 3-AT (Upper Left) and then restreaked onto SC–Trp–Leu–His with 3-AT medium (Upper Right) or an X-Gal indicator plate (Lower Left) to assess his3 and lacZ reporter gene activities, respectively. (Lower Right) Yeast strain Y190 was cotransformed with different combinations of plasmid constructs: +, cotransformant of GAL4bd and GAL4ad vector, which carries yeast trp1 and leu2 markers respectively; +, GAL4bd-murine p53 and GAL4ad-SV40 large T antigen fusion constructs, which serve as the positive control; E-cadCD + β-cat, GAL4bd–E-cadherinCD and GAL4ad–β-catenin fusion constructs; α-cat + β-cat, GAL4bd–α-catenin and GAL4ad–β-catenin fusion constructs; α-cat + E-cadCD, GAL4bd–α-catenin and GAL4ad–E-cadherinCD fusion constructs.](image-url)
galactoside as a chromogenic substrate. Each assay was performed in triplicate with three independent clones; the mean and standard deviation were then calculated. Cotransformsants of E-cadherin–β-catenin, α-catenin–β-catenin, and p53–SV40 large T antigen had enzymatic activities of 69.4 ± 29.7, 75.7 ± 26.9, and 5.81 ± 2.3 nmol/min per mg of protein extract, respectively. However, cotransformsants of GAL4bd/GAL4ad and α-catenin–E-cadherin expressed enzymatic activities of only 0.38 ± 0.08 and 0.29 ± 0.18 nmol/min per mg of protein extract, respectively.

Since different promoter sequences were inserted upstream of each reporter gene in Y190, transcriptional activation of both genes represents independent confirmation of the specificity of protein–protein interactions. Therefore, pairwise expression of the cadherin–catenin complex proteins in yeast allows us to determine unequivocally the hierarchy of protein–protein interactions: E-cadherin ↔ β-catenin ↔ α-catenin.

Defining the Hierarchy of Protein–Protein Interactions in the Cadherin–Catenin Complex by an in Vitro Protein Binding Assay. We examined interactions between E-cadherin and catenins by in vitro binding in whole-cell extracts using a GST fusion protein with E-cadCD (12). [35S]Methionine/cysteine-labeled cell lysates from the following cell lines were used for this analysis: MDCK cells, which express cadherin–catenin complexes that have been recently characterized (13); PC9 cells that do not express α-catenin and do express E-cadherin, β-catenin, and plakoglobin (8); and colon carcinoma cells (HCT116 and SW480), which express E-cadherin, α-catenin, β-catenin, and plakoglobin as shown below.

Proteins of apparent molecular masses of 102 kDa, 94 kDa, and 86 kDa were specifically bound by GST–E-cadCD from extracts of MDCK, HCT116, and SW480 cells (Fig. 2 Upper). The pattern of bound proteins resembles that of the catenins coimmunoprecipitated with E-cadherin when using E-cadherin antibodies (7). In Fig. 2 Lower, the identities of bound proteins as α-catenin, β-catenin, and plakoglobin were confirmed by immunoblotting with specific antibodies to each protein. In addition, we found that another armadillo family member, p120, interacted specifically with GST–E-cadCD (Fig. 2 Lower).

In extracts of PC9 cells, which do not contain α-catenin, we found that β-catenin, plakoglobin, and p120 bound specifically to GST–E-cadCD (Fig. 2 Lower, lanes 10, 12, and 14; see also ref. 8). In some cases (e.g., Fig. 2 Lower, lanes 2, 6, and 12), proteins with electrophoretic mobilities faster than the respective catenins were detected; these proteins appear to be degradation products even though precautions against proteolysis were taken (see Materials and Methods). These results support a conclusion from our genetic analysis, that E-cadherin cytoplasmic domain binds directly to β-catenin in the absence of α-catenin.

There are differences in the relative amounts of α-catenin, β-catenin, and plakoglobin bound to GST–E-cadCD from different cell extracts (Fig. 2 Upper). We sought to determine whether this was due to variations in levels of proteins expressed in different cell lines or differences in the accessibility of catenins to GST–E-cadCD. In immunoblot analysis, HCT116 and SW480 cells expressed similar amounts of α-catenin and plakoglobin, but SW480 cells expressed ~3-fold more β-catenin than did HCT116 cells (data not shown). To examine whether pools of catenins existed that had different capacities for binding GST–E-cadCD, we fractionated cell lysates by FPLC gel filtration in Superose 6 (Fig. 3). Fractionation of a SW480 cell extract showed that cadherin eluted in a single sharp peak with an apparent molecular mass of ~1,000–2,000 kDa (peak A). α-Catenin (data not shown) and β-catenin (Fig. 3 Upper) were eluted with a bimodal distribution; ~95% of α-catenin (data not shown) and β-catenin (Fig. 3 Upper) were eluted in a fraction with an apparent molecular mass of ~220 kDa (peak B), and the remainder was eluted in the same fractions that contained cadherin (peak A, Fig. 3 Upper). The elution profile of cadherin from HCT116 cell extracts was similar to that from SW480 cells. However, the elution profiles of α- and β-catenin were notably different from the profiles in SW480 cells. Eighty percent of both α-catenin (data not shown) and β-catenin (Fig. 3 Lower) in the HCT116 cell extract was eluted in the same peak A fractions that contained E-cadherin, and

![Figure 2](image2.png)

**Fig. 2.** (Upper) In vitro binding assay of E-cadCD and [35S]methionine/cysteine-labeled cell lysate. Extracts from HCT116, SW480, and MDCK cells were incubated with GST alone (lanes 1, 3, and 5) or GST–E-cadCD fusion protein bound to glutathione-agarose beads (lanes 2, 4, and 6). Proteins were separated in SDS/7.5% PAGE gels, and the fluorograms are shown. The arrowheads denote the relative electrophoretic mobilities of α-catenin, β-catenin, and plakoglobin (top to bottom). The relative electrophoretic mobilities of molecular weight marker proteins are shown on the right. (Lower) Immunoblots of proteins from cell lysates of SW480 (lanes 1–8) and PC9 cells (lanes 9–14) bound to GST (lanes 1, 3, 5, 7, 9, 11, and 13) or GST–E-cadCD fusion protein (lanes 2, 4, 6, 8, 10, 12, and 14). Immunoblots were probed with antibodies specific for α-catenin (lanes 1 and 2); β-catenin (lanes 3, 4, 9, and 10); plakoglobin (lanes 5, 6, 11, and 12); and p120 (lanes 7, 8, 13, and 14). Arrowheads denote the relative migration of the appropriate catenin in these SDS/PAGE gels. Note there is an additional protein that migrates between α- and β-catenin. This protein has been found before in cadherin–catenin immunoprecipitates (18, 19), but its identity remains unknown.

![Figure 3](image3.png)

**Fig. 3.** Fractionation of cadherin and catenins by FPLC of SW480 and HCT116 cell lysates. (Upper) Cadherin and β-catenin immunoblots of SW480 cell lysate fractions. (Lower) Cadherin and β-catenin immunoblots of HCT116 cell lysate fractions. An aliquot of each fraction was incubated with GST–E-cadCD protein bound to glutathione-agarose beads; the beads were washed, boiled in SDS sample buffer, separated in SDS/7.5% PAGE gels, and processed for immunoblotting with β-catenin antibody. Numbers at the top of the immunoblots refer to column fractions; void volume (fraction 0). The column was calibrated by using protein standards that eluted in the following fractions: thyroglobulin (M, = 669,000; fraction 10); apoferritin (M, = 443,000; fraction 13); β-amylase (M, = 200,000; fraction 15); alcohol dehydrogenase (M, = 150,000; fraction 17); bovine serum albumin (M, = 66,000; fraction 17); and carbonic anhydrase (M, = 29,000; fraction 25).
The elution profile of plakoglobin was not examined.

Analysis of interactions of cadherin and catenins in peak A fractions by immunoprecipitation revealed an endogenous complex of these proteins (data not shown). Assuming that the molar ratio of proteins in the cadherin–catenin complex is 1:1:1 E-cadherin/β-catenin/α-catenin (13, 14, 20), we would expect a complex of these proteins to have an apparent molecular mass of ≈350 kDa rather than ≈1,000–2,000 kDa. However, since cell lysates were fractionated in Superose 6 in buffer containing detergent, we cannot draw conclusions about the absolute molecular mass of complexes separated under these conditions. Nevertheless, the fact that catenins fractionated in a bimodal distribution suggests that catenins exist in at least two different pools.

To test for the interaction of different pools of catenins with exogenous GST–E-cadCD, we performed in vitro binding assays using peak A and B Superose 6 column fractions. From HCT116 (Fig. 3 Lower) and SW480 (data not shown) cells, only β-catenin that eluted in peak B fractions bound GST–E-cadCD. This result supports the general conclusion that E-cadCD interacts directly with β-catenin. In contrast, β-catenin from fractions composing peak A did not bind GST–E-cadCD. Since the large molecular weight pool of β-catenin is bound to endogenous E-cadherin, these results indicate that β-catenin bound to one E-cadherin molecule cannot bind to a second cadherin. In contrast, β-catenin in peak B fractions is accessible to exogenous E-cadherin. This implies that this pool of β-catenin is not excluded from the endogenous E-cadherin–catenin complex because it is not competent to bind to E-cadherin but rather that it constitutes a separate catenin pool. Partitioning of β-catenin into different protein pools may be important for the regulation of cadherin–catenin complex assembly (13, 14) and the binding of β-catenin to other proteins such as APC (adenomatous polyposis coli) and EGF (epidermal growth factor) receptor (18, 19, 21).

To investigate interactions between β-catenin and armadillo family members in cell extracts, we expressed histidine-tagged α-catenin in E. coli and tested its binding to proteins in detergent extracts of MDCK cells. β-Catenin and plakoglobin bound to histidine-tagged α-catenin (Fig. 4). Significantly, neither E-cadherin nor p120 were detectably bound to α-catenin fusion protein. This finding confirms biochemically that E-cadherin and α-catenin do not interact directly and that β-catenin binds to α-catenin.

These binding assays revealed an unexpected difference between β-catenin, plakoglobin, and p120, all of which are structurally related (22). p120 bound to GST–E-cadCD (Fig. 2 Lower) but not to histidine-tagged α-catenin (Fig. 4). However, other members of the armadillo family, β-catenin and plakoglobin, bound both GST–E-cadCD and histidine-tagged α-catenin (Fig. 4). These findings imply structural and functional differences between p120 and other armadillo gene family members. We note, however, that p120 could be immunoprecipitated from both peak A and B fractions with α-catenin using an α-catenin antibody (data not shown). It is possible that posttranslational modifications of α-catenin or additional proteins are required for formation of p120/α-catenin interactions.

Mapping β-Catenin Binding Domains in Proteins of the Cadherin–Catenin Complex by Using the Yeast Two-Hybrid System. Since we found strong agreement between protein–protein interactions obtained with the yeast two-hybrid system and direct binding assays with recombinant proteins, we sought to define domains of E-cadherin and α-catenin that are required for binding β-catenin and, therefore, the formation of the cadherin–catenin complex. We constructed deletion mutants within the E-cadherin cytoplasmic domain and in α-catenin in the GAL4 DNA binding domain vector and transformed them pairwise into Y190 with constructs used previously to test for activation of reporter genes (Fig. 5). In contrast to the E-cadherin/β-catenin cotransformant that survived selection on SC–Trp–Leu–His containing 3-AT, a cotransformant of β-catenin and E-cadCD(−25), which contains a 25-amino acid deletion from position 815 to 839 in the cytoplasmic domain of E-cadherin, did not grow. This yeast transformant also failed...
to activate the β-galactosidase reporter gene, as shown by the lack of blue-colored colonies (Fig. 5) and the absence of β-galactosidase activity in the quantitative colorimetric assay; specific enzymatic activity was measured as 0.49 ± 0.26 nmol/minute per mg of protein extract from three independent clones. We conclude that the 25-amino acid deletion from E-cadCD is a binding site for β-catenin. This confirms a recent analysis in transfected fibroblasts that this region of E-cadherin is a catenin-binding site (11), although we show here that this is a binding site for β-catenin.

We next investigated domains of α-catenin involved in binding β-catenin. Using the yeast two-hybrid system, we found that an α-catenin construction with a deletion of the N-terminal 606 amino acids did not interact with β-catenin (LacZ activity with 0.34 ± 0.15 nmol/min/mg of protein when this construct was cotransformed into Y190 with GAL4bd-β-catenin), while an α-catenin construction with a C-terminal deletion from position 607 to 906 still bound β-catenin [LacZ activity was 148.2 ± 31.6 nmol/min/mg of protein when GAL4bd-α-catenin(N) was cotransformed with GAL4ad-β-catenin fusion construct] (Fig. 5). We also examined whether α-catenin formed homodimers, as suggested by its sequence homology to vinculin (23). We found that neither the his3 nor lacZ reporter genes were activated by cotransformation of plasmids containing α-catenin fused to both the GAL4bd and GAL4ad vectors (LacZ activity was 0.49 ± 0.14 nmol/min/mg of protein extract from three independent clones) (Fig. 5).

These results reveal that binding sites for β-catenin are located in the cytoplasmic domain of E-cadherin and the amino-terminal domain of α-catenin, respectively. Analysis of the amino acid sequences of these two β-catenin-binding domains deduced from cDNAs reveals little or no homology. Although we have not determined the kinetics of protein binding, it is noteworthy that α-catenin can be removed from the E-cadherin–catenin complex with octylglucoside (7) or high salt (13), whereas β-catenin cannot. This suggests differences in the molecular interaction between binding sites for β-catenin on E-cadherin and α-catenin, respectively.

Defining the order of protein–protein interactions in the E-cadherin–catenin complex is important for understanding the structure, function, and regulation of the assembly of this complex. To determine protein–protein interactions in the cadherin–catenin complex, previous studies have used specific antibodies to deplete one component from the cadherin–catenin complex and study interactions between the remaining molecules (13, 14). Alternatively, cadherin–catenin interactions in cell lines that lack one component of the complex, such as the α-catenin-deficient PC9 cells, have been examined (8). Nevertheless, a caveat to those studies is that other proteins may affect cadherin–catenin interactions. The results presented in this study show a synergistic approach to this problem using a combination of a yeast genetic assay and a biochemical assay. Our data reveal that the trimolecular complex has the following binding order of proteins: E-cadherin ↔ β-catenin ↔ α-catenin. A small region of the cytoplasmic domain of E-cadherin binds to β-catenin, and β-catenin binds to the amino terminus of α-catenin, while E-cadherin does not bind directly to α-catenin. Since evidence shows that β-catenin is at the convergence of several signal transduction pathways involving Src tyrosine kinase (24–26) and extracellular signaling molecules (epidermal and hepatocyte growth factors and Wnt-1) (19, 27–29), the finding that β-catenin is the linker protein between E-cadherin and α-catenin strengthens the hypothesis that β-catenin plays a pivotal role in regulating the structural organization and function of the cadherin–catenin complex.

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