α-Catenin Can Form Asymmetric Homodimeric Complexes and/or Heterodimeric Complexes with β-Catenin*

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The cadherin-based transmembrane cell-cell adhesive complex is thought to be composed of a cadherin molecule, a β-catenin, and an α-catenin, which connects the complex to the cytoskeleton. The precise stoichiometry of this complex remains uncertain. We have used a series of recombinant molecules and biophysical techniques to assess the multimeric state of human α- and β-catenin in vitro and then visualized them by electron microscopy after rotary shadowing. Calculated solution molecular masses are 213 kDa for α-catenin, 78 kDa for β-catenin, and 186 kDa for both. This suggests that α-catenin exists as a homodimer in solution, β-catenin is a monomer, and when both are present, they form αβ-catenin heterodimers. Co-precipitation and surface plasmon resonance assays localize the site of α-catenin dimerization to the NH2-terminal 228 amino acids. This region encompasses a high-affinity (Kd = 100 nM) binding site for β-catenin that lies between residues 54 and 157. We anticipate that the oligomeric state of α-catenin and the relative stoichiometry of the components in the membrane adhesion complex will be dynamic and regulated by β-catenin, cell adhesion, and probably other factors as well.

E-cadherin is a 120-kDa transmembrane glycoprotein whose calcium sensitive homotypic adhesion mediates epithelial cell-cell adhesion, cell polarization, and other differentiation events (2). Two proteins, called α-catenin and β-catenin, closely associate with the cytoplasmic tail of E-cadherin and connect the membrane adhesion complex to the cytoskeleton (3, 4). While it is generally appreciated that β-catenin binds both E-cadherin and α-catenin, the precise molecular details of the adhesion complex and the oligomeric state of the proteins involved remains uncertain. The crystal structure of the first extracellular domain (EC-1) of N-cadherin reveals a dimeric configuration (5), and recombinant fragments of C-cadherin also form dimers, a configuration required for productive adhesion (6). It therefore seems likely that the active state of cadherin is dimeric.

The oligomeric state of the other components of the complex is more controversial. Heterodimeric complexes of α-catenin and β-catenin at a 1:1 ratio have been detected by immunoprecipitation (7, 8) and supported by yeast two-hybrid assays that detect a direct interaction between α- and β-catenin (9). In these assays, neither α-catenin or β-catenin showed evidence for homodimerization. Yet, in studies assessing the interaction of α-catenin with F-actin, 1 catenin molecule was bound for every 7 actins (3). Since F-actin exists as a double helical filament with 7 molecules per strand or 14 molecules per turn of the helix, we proposed that at saturation 2 α-catenins bound per turn of the helix, and suggested that α-catenin may exist as a dimer in solution.

In the present study, we examine the oligomeric state of both α-catenin and β-catenin in solution, as well as the state of heterocomplexes of α- and β-catenin. We find that while β-catenin consistently exists as a monomer under the solution conditions examined, α-catenin is dimeric. When β-catenin binds, the α-catenin homodimer is partially dissociated, yielding heterodimeric complexes of α- and β-catenin. These results suggest the presence of a complex interaction between β-catenin and α-catenin homodimers that might include at least two distinct functional states.

**EXPERIMENTAL PROCEDURES**

**Preparation and Purification of Recombinant Proteins—Full-length α- and β-catenin clones were as described previously (1, 3). Constructs were prepared using the pGEX (Pharmacia Biotech Inc.) prokaryotic expression vectors subcloned by either polymerase chain reaction or restriction enzyme digestion of the full-length clone and were expressed as glutathione-S-transferase (GST) fusion proteins in *Escherichia coli*. For some constructs, the pGEX-KT vector was also utilized to facilitate thrombin cleavage of the recombinant fusion protein from GST (10). Bacterial cultures were grown for 3 h and then induced for 3 h with isopropyl-β-D-thiogalactopyranoside (0.1 mM) before harvesting. Lysis was achieved by four repetitions of a 50-s sonication on ice in TBSE (20 mM Tris, pH 8, 150 mM NaCl, 1 mM EDTA) with 1 mM DTT and 1 mM each of the protease inhibitors PefablocSC (Centrachem), chymostatin, leupeptin, antipain, and pepstatin (all from Sigma). The 15,000 × g supernatant of the lysate was affinity purified on glutathione-agarose (Sigma) at 4 °C and washed extensively with TBSE containing 0.1 mM glutathione, pH = 8.0 (Sigma) in TBSE containing PefablocSC and 1 mM DTT. Alternatively, GST was cleaved from the peptides while the peptides were bound to the GST affinity column by the addition of 10 ml of 20 mM Tris, 120 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 25 mM CaCl2, 2.5 mM KCl at 20 °C for 10 min. After digestion, the cleaved peptide was eluted, and 20 mM DTT was added to the eluates, which were stored on ice for at least 1 h. Eluted recombinant proteins were then dialyzed into Tris-buffed binding buffer (TBBB, 20 mM Tris, pH 7.5, 100 mM NaCl, 0.1 mM DTT) prior to further use.

**Gel Filtration Chromatography—**To determine the Stokes' radius,
thrombin-cleaved fusion proteins (500–1500 M) were fractionated at 20 °C in TBBB using a 16/60 HiPrep® Sephacryl S-300 high resolution column (Pharmacia). The column was calibrated using thyroglobulin, apoferritin, catalase, aldolase, alcohol dehydrogenase, and BSA (all from Sigma) as standards. Collected constant volume fractions were analyzed by SDS-PAGE, and the protein in each fraction quantified by densitometry after Coomassie Blue staining using a UMAX Vista-S6E gel scanner and the ScanAnalysis™ (Biosoft) software package on an Apple Macintosh® Computer.

**Sedimentation Experiments**—To determine the sedimentation velocity of the thrombin-cleaved fusion peptides, 5–20% sucrose gradients in 20 mM Tris, 100 mM NaCl, 0.1 mM DTT were made using a 12-mL gradient maker (Hoefer) and layered into tubes using a HBI Auto-Densi-Flow™ 12c gradient layering and harvesting device. The protein samples (250–750 M) were layered on top of the sucrose gradients, and the tubes were spun at 200,000 g for 11–12 h at 20 °C. Fractions (0.26 mL) were collected and analyzed by SDS-PAGE as above.

**Estimation of Solution Molecular Weight**—The solution molecular weights were estimated by using size chromatography to determine the Stokes radius (Rg) followed by velocity sedimentation to determine S values. Molecular weight was calculated from the Svedberg equation by calculating a diffusion coefficient from the Stokes-Einstein equation, and then using the measured Stokes radius to calculate the molecular weight from the measured sedimentation velocity. The value of (1 − ηp) was determined empirically from the average of 3 standards of known size. Specifically,

\[ D = \frac{RT}{6\pi\eta NR^2} \]  

(Stokes-Einstein equation) (Eq. 1)

and

\[ M_s = \frac{RTS}{D(1-\eta p)} \]  

(Svedberg equation) (Eq. 2)

where D is the diffusion coefficient; R, the universal gas constant; T, the temperature in degrees Kelvin; N, Avogadro’s number; η, the viscosity of the solution; S, the sedimentation coefficient in Svedbergs; r, the partial specific volume of the protein (taken as 0.74 initially, ultimately determined empirically from standards); and ρ, the density of the buffer (taken as unity).

**Binding Studies Using Glutathione-Agarose Precipitation**—GST-containing fusion peptides (2 M), thrombin-cleaved fusion peptides (10 M), and 1.5% BSA were combined at 20 °C for 2–3 h in TBBB containing 1 mM MgCl₂, and 0.1% Triton X-100. The mixture was cooled briefly on ice, and then 50 g of a 50% slurry of glutathione-agarose in the same buffer was added to 200 g of the reaction mixture. After 20 min of incubation, the pellets were washed 4 times in 500 g of the same buffer, and analyzed by SDS-PAGE. Bound protein was quantified by densitometry as described above.

**Surface Plasmon Resonance Studies of α-Catenin / β-Catenin Interactions**—The interaction of α-catenin and β-catenin recombinant proteins was also assayed using surface plasmon resonance using a Pharmacia BIAcore® 2000 instrument (11). Purified recombinant GST free β-catenin was diluted to 10 M in 10 mM MES buffer (pH 5.0) and coupled to the sensor chip (Pharmacia Biosensor) using amine-coupling chemistry. Briefly, the dextran-coated surface of the sensor chip was activated for protein coupling with a 1:1 dilution of 40 M of N-hydroxysuccinimide, N-ethyl-N,N′-diethylenepropanoylcarbodiimide at 5 M/min, followed by the addition of recombinant β-catenin (using a range of concentrations). The coupling reaction was terminated by deactivation of the activated groups on the dextran surface by 10 M of 1 M ethanolamine. These conditions yielded sensor surfaces containing 200–1,000 resonance units. Changes in resonance were assessed after the addition of increasing amounts of recombinant α-catenin, following established instrumental procedures (Pharmacia Biosensor). Surfaces were regenerated after ligand binding by brief treatment with 50 mM NaOH until the resonance units detected returned to baseline levels. The surfaces so conjugated were stable for at least 100 binding-regeneration cycles. Rate constants were calculated using the BIAEvaluation 2.1 software package (Pharmacia Biosensor). Pt-C replicas were floated onto standard electron microscopy grids, examined, and photographed using a Zeiss EM10A. Digitized negatives were used to construct figure composites using Adobe Photoshop 3.0 operating on an Apple Macintosh 7100 computer.

**RESULTS**

α-Catenin Is a Homodimer in Free Solution—Recombinant polypeptides were prepared that collectively represented full-length human α- and β-catenin as well as a series of complementary peptides representing various sequences within α-catenin (Fig. 1). These polypeptides were used either as fusion peptides with GST, or more often as GST-free peptides after cleavage with thrombin. To determine the solution state of α- and β-catenin, full-length GST-free recombinant proteins were evaluated by gel filtration and sedimentation velocity. A representative gel filtration profile is shown (Fig. 2). Generally, standards were run separately from test samples, although the inclusion of standards simultaneously with the test samples did not affect outcome. In these experiments, it was apparent that α-catenin in TBBB buffer at 20 °C migrated more rapidly than did β-catenin. To determine the Rg of each species (Fig. 3), their elution positions were compared with a standard curve established using thyroglobulin (8.6 nm), catalase (5.2 nm), alcohol dehydrogenase (4.5 nm), and BSA (3.6 nm). Each determination was repeated between 2 and 5 times, and a separate standard curve was constructed for each catenin preparation. The Rg values derived from these determinations are summarized in Table I.

Similar methods were used for determination of the sedimentation values (Figs. 4 and 5). Standard sedimentation curves were constructed, using as standards thyroglobulin (19.5 S), catalase (11.3 S), alcohol dehydrogenase (7.6 S), BSA (4.5 S), and cytochrome c (1.8 S). A separate standard curve was constructed for each catenin preparation. The Svedberg coefficients derived from these determinations are summarized in Table I. Solution molecular weights were then estimated from these values as outlined under “Experimental Procedures.” These values are summarized in Table I. Comparison of the calculated solution molecular weights with the theoretical molecular weights based on their peptide sequences indicates that in solution, α-catenin exists predominately as a...
homodimer; β-catenin is monomeric; and that the major species in a mixture of α- and β-catenin is a heterodimeric complex composed of one α-catenin and one β-catenin, although other species are suggested by minor peaks in the sedimentation profile.

β-Catenin Binds to a Site Near the Homodimerization Site in α-Catenin—The sedimentation and gel filtration studies suggested that β-catenin binding competed with the homodimerization of α-catenin. To better understand this process, we used a series of recombinant polypeptides (Fig. 1) to define the minimal sites within α-catenin for both β-catenin binding and homodimerization. Various GST containing fusion polypeptides were incubated with full-length α-catenin; binding was assessed by the ability of glutathione-agarose to capture the full-length (GST-free) α-catenin. Fig. 6 shows that full-length α-catenin (migrating at about 100 kDa) co-sediments with fusion peptides encompassing the NH2-terminal 228 residues of α-catenin, but not with peptides representing any combination of residues distal to residue 229. These results suggest that dimerization of α-catenin is mediated by sequences proximal to residue 228. Consonant with this observation is the finding that α(E)-catenin, which arises by 3' alternative mRNA splicing of the α(E)-catenin gene (1), also appears to dimerize by a mechanism similar to that utilized by α(E)-catenin (Fig. 6, lane L). Controls are shown to prove the interaction is specific including lane A, which shows no nonfusion α-catenin sediments with glutathione-agarose beads in the absence of an interaction with a GST-fusion protein.

Similar techniques were used to identify the region of interaction with β-catenin (Fig. 7). No binding was seen with recombinants representing sequences distal to residue 229. The smallest sequence that bound β-catenin spanned amino acids 54–157. Interestingly, these residues, while sufficient to mediate the binding to β-catenin, were insufficient to mediate binding to α-catenin (i.e. homodimerization). Thus, it appears that whereas both homodimerization and β-catenin binding involve similar regions of α-catenin, the two activities are distinct.

α-Catenin Binds β-Catenin with High Affinity—Given the ability of β-catenin to compete effectively for the self-association of α-catenin, as well as the importance of this interaction for the formation of the membrane adhesion complex, the quantitative properties of this interaction were of considerable interest. The binding kinetics of full-length α-catenin interacting with immobilized β-catenin were thus measured by surface plasmon resonance, using a Pharmacia BIACore™ Biosensor 2000 instrument. The binding of six concentrations of α-catenin at pH 7.5 in TBBB was assessed using sensor surfaces containing between 500 and 2000 resonance units of bound β-catenin (see “Experimental Procedures”). Representative sensorgrams are presented in Fig. 8. Good fits to the sensorgrams were obtained using a simple bimolecular ligand binding model, as determined by the absence of systematic residuals and a linear $K_a$ versus $C$ plot (Fig. 8, inset). From these analyses, a $K_a$ of 100 nM was determined. Similar analyses were also attempted to determine the strength of the homodimerization site in α-catenin. These studies did not yield reliable binding isomers. We do not know whether this is a consequence of a weaker interaction mediating homodimerization, an artifact of the immobilization reaction, or an effect arising from competition between solution phase homodimerization versus binding to immobilized α-catenin.

The Morphologies of the Rotary Shadowed Molecules Are Consistent with Their Biophysical Properties—The ultrastructure of platinum/carbon replicas generated by rotary shadowing of purified recombinant α- and β-catenin was also evaluated (Fig. 9). In these studies, α-catenin was found to be a 35.8 ± 4.7 nm elongated molecule with an enlarged knob at one end. This structure is reminiscent of the structure of vinculin, a protein that shares sequence (13, 14) and functional homology with α-catenin (15). We believe the majority of the α-catenin prepared for imaging is monomeric, but some larger complexes appear (Fig. 9A, lower right box) that may represent dimers. These forms are less frequent. Representative images of β-catenin are shown in Fig. 9B. These molecules appear to be simple 13.7 ± 1.5 nm globular monomers with a tiny but consistent dot that is sometimes suggestive of a tail-like structure. The morphology of the α-catenin-β-catenin heterodimer is shown in Fig. 9C. The width of these complexes is 15.0 ± 1.8 nm, comparable to the diameter of the β-catenin molecule. The length is 21.0 ± 2.4 nm, suggesting some conformational change accompanying heterodimerization. The schematic below each part of the figure shows our conception of the general shape of each molecule and how they might fit together.

DISCUSSION

The results reported here establish that: 1) α-catenin exists in a dynamic monomeric-dimeric equilibrium with the dimeric state being favored when the molecule is in pure solution at micromolar concentrations; 2) the dimerization site is confined to the amino-terminal 228 residues; 3) β-catenin binds α-cate-
**α-Catenin Interactions**

### TABLE I

| Protein          | Stokes radius | n | Sedimentation velocity | n | Calculated molecular weight | Proposed solution state | Theoretical molecular weight |
|------------------|---------------|---|------------------------|---|----------------------------|-------------------------|-------------------------------|
| α-Catenin        | 7.40 ± 0.385² | 4 | 7.04 ± 0.472²          | 4 | 213,000                    | Homodimer                | 200,560                       |
| β-Catenin        | 4.62 ± 0.531² | 4 | 3.86 ± 0.266²          | 5 | 73,000                     | Monomer                 | 85,505                        |
| α-Catenin · β-catenin | 6.82 ± 0.255² | 2 | 6.38 ± 0.367²          | 5 | 178,000                    | Heterodimer              | 185,785                       |

* Average based on number of determinations (n) ± 1 S.D.

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**FIG. 4. Sucrose gradient sedimentation of the catenins.** Each trace indicates the amount of α-catenin (○), β-catenin (□), α-catenin in the mixed αβ-catenin sample (●), or β-catenin in the mixed αβ-catenin sample (○) as determined by scanning densitometry. The upper gel is α-catenin; the middle gel is β-catenin; and the lower gel is the mix with the upper band indicating α-catenin, and the lower band indicating β-catenin. Absorbency is in arbitrary units.

**FIG. 5. Estimation of the sedimentation coefficient of the catenins.** The relationship of how each catenin sedimented in the sucrose gradient with respect to markers of known S value is shown. Markers were: thyroglobulin (19.5 S); catalase (11.3 S); alcohol dehydrogenase (7.6 S); BSA (4.3 S); and cytochrome c (1.8 S). The best fit derived from five standards is shown. The calculated sedimentation value for each catenin is shown for a representative experiment. Based on five determinations, the Svedberg coefficient for α-catenin was 7.0 ± 0.47 S; β-catenin, 3.9 ± 0.27 S; and the α-catenin-β-catenin complex, 6.4 ± 0.37 S. All determinations were made with the nonfusion recombinant proteins.

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**FIG. 6. Co-sedimentation assays localize the region of interaction for dimerization of α-catenin to the NH₂-terminal 228 amino acids.** GST fusions with various fragments of α-catenin were co-precipitated with glutathione-agarose beads in the presence of non-dimerization fragments. Lane A shows the normal migration of recombinant α-catenin after cleavage to remove the GST peptide (arrow). Lane A shows the pellet from sedimentation with glutathione-agarose beads in the presence of only non-dimerization fragments. Lanes B–J are non-dimerization fragments co-sedimented with α-catenin fusions N1–95 (B), 96–226 (C), 154–157 (D), 154–226 (E), N1–228 (F), 1229–462 (G), N1–576 (H), C461–907 (I), and the full-length α-catenin-GST fusion (J). Lane K shows that the α(E)-catenin isoform also co-sediments forming mixed isoform dimers. Lane L is a full-length β-catenin GST fusion included as a positive control in each run.

**FIG. 7. Co-sedimentation assays identify the binding site for β-catenin.** GST fusion polypeptides with various fragments of α-catenin were co-precipitated with glutathione-agarose beads in the presence of full-length β-catenin. Lane M shows the normal migration of recombinant β-catenin after cleavage to remove the GST peptide (arrow). Lane A shows the pellet from sedimentation with glutathione-agarose beads in the presence of only non-dimerization fragments in β-catenin to show that this protein does not sedimate in the presence of glutathione-agarose. Lanes B–J are non-dimerization fragments co-sedimented with β-catenin fusions N1–95 (B), 96–226 (C), 154–157 (D), 154–226 (E), N1–228 (F), 1229–462 (G), N1–576 (H), C461–907 (I), and the full-length α-catenin-GST fusion (J). Lane K is full-length β-catenin-GST fusion to confirm that no homotypic interaction is seen between β-catenins.

The order of protein interactions has been difficult to ascertain. The two-hybrid assay (9), differential extraction assay (8), and gel overlay assays (16) have sought to address this question. Some interactions, including the archetypal cadherin interaction, include β-catenin, α-catenin, and a cadherin molecule in the complex, while others, including fascin (17) or the transcription factor LEF (18), have suggested that β-catenin binds its substrate in the absence of α-catenin. This may be due to competition for the same site, or possibly other reasons.
Fig. 8. α-catenin binds β-catenin with high affinity. Biosensor tracings of a representative experiment reporting the binding of nonfusion full-length recombinant α-catenin to immobilized β-catenin. The sensograms represent increasing concentrations (C) of α-catenin (0.2, 0.4, 0.6, 0.8, 1.0, 2.0 μM, respectively, for curves A–F). These curves were analyzed in both association and dissociation phases for the kₐ and kₘ rates. A secondary plot (inset) of kₐ versus C was then generated to calculate Kᵦ. For these experiments, an overall Kᵦ = 100 nM was determined for the interaction of α-catenin with β-catenin.

Fig. 9. Rotary shadowed images of α-catenin, β-catenin, and the αβ-catenin heterodimer. A gallery of 12 representative examples of α-catenin (A), β-catenin (B), and a mix of both species (C) is shown (original magnification = 40,000×; bar = 40 nm). The images demonstrate the elongated shape of α-catenin with an enlargement at one end. The β-catenin molecule appears to be globular with a small bright internal structure. The mix shows a heteromeric complex of two globular structures, one with internal features similar to that seen in β-catenin. The “tail” of α-catenin appears to be obscured by the β-catenin molecule. Beneath each gallery, a schematic illustrates this model.

Having to do with the role of β-catenin as a signal transduction molecule. Another example of the independent function of α-catenin and β-catenin is the recent work on the β-catenin-APC complex that suggests that the microtubule binding functions of APC may provide a connection of that complex to the cytoskeleton (19). This work finds no association of the α-catenin/actin interaction in this complex and provides no explanation or localization for the initially identified coprecipitating α-catenin molecule (20). The fact that β-catenin can be identified in conditions where it does not interact with α-catenin suggests the possibility of the converse as well. It is possible that there are conditions in which α-catenin is present as a dimer and not bound to β-catenin.

Determination of the minimal binding site by the use of progressively smaller recombinants is a valuable technique but has certain inherent limitations. In this study, the NH₂-terminal region of α-catenin defines a 228 residue domain that is difficult to divide with respect to the dimerization site. Both the peptide containing amino acids 1–96 and that containing amino acids 97–226 co-sediment full-length α-catenin with apparent lower affinity, suggesting a complex interaction site. This might be explained by a tertiary structure in this domain where the protein folds back on itself to produce a dimerization site with components from both amino acids 1–96 and 97–226.

The β-catenin binding site appears to also illustrate division of a binding site as less binding is seen to peptides 1–96 and 97–226 than is seen to a peptide encompassing residues 54–157. We interpret this result as suggesting that the β-catenin binding site centered around residue 96 and that flanking sequences, each retain a portion of the binding site and are still capable of binding β-catenin albeit with lower affinity. It is also clear that while the sites of interaction for homodimerization and β-catenin binding nearly overlap, they are nevertheless distinct. While β-catenin binding can be mapped to a relatively small region immediately surrounding amino acid 96, the α-catenin homodimerization site is more diffuse. Both the dimerization and β-catenin binding sites do not overlap with other α-catenin interaction sites, including the C-terminal actin binding domain (3) and a central region of the molecule where an interaction with α-actinin has been proposed (21). Note that while this work was under review, two studies appeared showing that the binding site for β-catenin on α-catenin was in the same region discussed above (21, 24).

Although the predominant species seen in mixes of α-catenin and β-catenin has a calculated molecular mass around 180 kDa, which suggests a heterodimer, other species may exist. Both the elution profile of the gel filtration column and the sucrose gradient fractionation show shoulders on the primary peaks. A representative example of this is seen in the sucrose gradient fractionation in Fig. 4. A clear second peak is seen in the α-catenin profile derived from quantification of the mixed sample. Although less pronounced, a shoulder can also be seen at the same fraction in the quantification of β-catenin. When fit to the curve, this suggests a possible second species with a sedimentation value of around 8.5 S. It is tempting to calculate a solution molecular weight for this species, however, the corresponding Stokes radius is difficult to ascertain. Although Fig. 2 shows an example of a shoulder peak in the quantification of α-catenin as part of the analysis of the mixed sample, that peak corresponds with the major peak for α-catenin in solution by itself. Thus it is as likely that this peak represents α-catenin dimer as another mixed species. The exact nature of higher order complexes is difficult to ascertain. They may be dependent on assay conditions like ionic strength and temperature. Although not shown, a smaller population of larger forms are seen by electron microscopy, which is consistent with the chromatographic data suggesting that higher order structures may be present. Overall, these findings suggest that in cells, dimers and other higher order structures may exist.

A central question is the implications of these findings for
the dynamics of adhesion complex assembly in vivo. While not directly addressed in these studies, several considerations emerge. Clearly, a dynamic equilibrium exists between α-catenin homodimerization and αβ-catenin heterodimers. In addition, the affinity of β-catenin for α-catenin is high. These findings suggest that the free levels of unassociated α-catenin in a cell will be low, at least in the absence of other factors that might regulate this interaction. This prediction has generally been supported by available evidence, although it must be considered that the published literature has emphasized the co-localization of α- and β-catenin rather than treating them as distinct populations. In addition, methods that could potentially distinguish free α-catenin pools, such as sizing chromatography and sucrose gradient sedimentation of whole cell extracts (22, 23), may be complicated by the similar migration profiles of α-catenin dimers and α-cateninβ-catenin complexes (Figs. 2 and 4). Recent studies suggest that functional adhesion requires clustering of the cadherins (25). A population of α-catenin homodimers may contribute to the dynamics of adhesion complex assembly and regulation by facilitating this clustering by dimerization or some combination of multimerization and interactions with α-actinin (21), actin (3), or spectrin. Like β-catenin, α-catenin is emerging as a complex multifunctional molecule that may participate in a range of binding interactions.

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