The Cytoskeletal Protein α-Catenin Unfurls upon Binding to Vinculin

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Adherens junctions (AJs) are essential for cell-cell contacts, morphogenesis, and the development of all higher eukaryotes. AJs are formed by calcium-dependent homotypic interactions of the ectodomains of single membrane-pass cadherin family receptors. These homotypic interactions in turn promote binding of the intracellular cytoplasmic tail domains of cadherin receptors with β-catenin, a multifunctional protein that plays roles in both transcription and AJs. The cadherin receptor-β-catenin complex binds to the cytoskeletal protein α-catenin, which is essential for both the formation and the stabilization of these junctions. Precisely how α-catenin contributes to the formation and stabilization of AJs is hotly debated, although the latter is thought to involve its interactions with the cytoskeletal protein vinculin. Here we report the crystal structure of the vinculin binding domain (VBD) of α-catenin in complex with the vinculin head domain (Vh1). This structure reveals that α-catenin is in a unique unfurled mode allowing dimer formation when bound to vinculin. Finally, binding studies suggest that vinculin must be in an activated state to bind to α-catenin and that this interaction is stabilized by the formation of a ternary α-catenin-vinculin-F-actin complex, which can be formed via the F-actin binding domain of either protein. We propose a feed-forward model whereby α-catenin-vinculin interactions promote their binding to the actin cytoskeleton to stabilize AJs.

Cell-cell contacts require the formation and stabilization of multiprotein cell membrane complexes coined adherens junctions (AJs). These complexes are directed by homotypic interactions of single-pass transmembrane receptors (cadherins) that have tandem calcium binding ectodomains and short but functionally critical intracellular tail domains (1). Cadherin interactions promote interactions of their tail domains with β-catenin, an oncoprotein that both functions as a component of AJs and regulates the activity of the T-cell transcription factor (TCF) family of transcription factors (2, 3). Interestingly, the cadherin-β-catenin complex is hardwired as they bind to one another immediately following their synthesis and are co-transported to the cell membrane as a nascent complex (4). Once there, β-catenin binds to the cytoskeletal protein α-catenin to form the functional ternary cadherin-β-catenin-α-catenin complex, and all three components are required for development and tissue homeostasis (5–8). Stabilization of AJs had long been thought to rely on the interaction of this ternary complex with F-actin, where increased pools of α-catenin at nascent junctions lead to the formation of α-catenin homodimers that bind to F-actin via a C-terminal domain (9, 10). However, the isolated ternary complex, or these complexes in the context of isolated cell membranes, cannot bind to F-actin (11, 12). Further, in these experimental systems, F-actin binding of the ternary complex is not restored by the addition of the cytoskeletal proteins vinculin or α-actinin that themselves can bind to F-actin (2). Rather, the function of the ternary AJ complex was suggested to impair the activity of the F-actin nucleating, branching Arp2/3 complex (13, 14). Finally, to add more complexity, other α-catenin binding partners, specifically Eplin (15) or vinculin (16, 17), have been suggested to play important roles in stabilizing AJs, as for example, vinculin knockdown disables cadherin receptor-actin connections and provokes marked reductions in AJs (18).

α-Catenin is a highly conserved, 906-residue cytoskeletal protein that is composed of four functional domains: (i) an N-terminal β-catenin interacting motif; (ii) a vinculin and α-actinin interacting domain; (iii) an I-afadin binding module that lies within a dimerization domain; and (iv) a C-terminal domain that can bind to both zonaula occludens-1 (ZO-1) and F-actin (19). The crystal structure of the N terminus of α-catenin, and of this domain (residues 57–264) in complex with an α-helix of β-catenin, revealed that this region is a homodimer composed of two antiparallel four-helix bundle domains and that β-catenin binds to α-catenin via a helix exchange mechanism (20). In addition, the crystal structure of the "M (modulation) fragment" (residues 377–633) (9, 21), which harbors the bind-
α-Catenin-Vinculin Complex Crystal Structure

Expressed proteins were purified using a HisTrap chelating nickel affinity column (GE Healthcare) and eluted over a gradient to 500 mM imidazole. Affinity tags were not cleaved for α-catenin 82–906 and 144–906 constructs. Further purification was performed on a Superdex 200 (GE Healthcare) in 20 mM Tris-HCl (pH 8), 150 mM NaCl, and 5 mM DTT and concentrated to about 25 mg/ml.

For crystallizations, cell pellets of His-tagged Vh1 and GST-tagged α-catenin VBD were lysed together in 20 mM Tris-HCl (pH 8) and 150 mM NaCl and purified on a GST-Sepharose column (GE Healthcare). The complex was eluted with 15 mM reduced glutathione, GST cleaved with PreScission protease in 20 mM Tris-HCl (pH 8), 150 mM NaCl, 1 mM EDTA, and 1 mM DTT, reloaded onto a GST-Sepharose column to remove the cleaved GST, and further purified on a preparative Superdex 200 26/60 column (GE Healthcare) in 20 mM Tris-HCl and 150 mM NaCl. The complex was concentrated to 27 mg/ml.

α-Catenin-Vinculin Crystallization—Vh1-VBD complex crystals were obtained by hanging drop vapor diffusion equilibration against a reservoir condition containing 12% (w/v) polyethylene glycol 3350 and 0.3M sodium acetate. The crystals were flash-frozen in liquid nitrogen and cryoprotected with 25% (v/v) glycerol.

X-ray Data Collection, Reduction, Structure Determination, and Crystallographic Refinement—x-ray diffraction data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) beamline BM22 at the Advanced Photon Source, Argonne National Laboratory and integrated and scaled using autoPROC (29), which uses XDS (X-ray Detector Software) (30) and SCALA (31) as the data reduction engine. The Vh1 domain of the vinculin-IpaA complex (32) was used as the search model to obtain molecular replacement solution with MOLREP (33). The difference electron density map calculated after initial rigid body refinement followed by restrained refinement with BUSTER (34) showed clear electron density for all four α-catenin α-helices, which enabled the manual building of the remaining model using COOT (Crystallographic Object-oriented Toolkit) (35). The final refinement statistics are detailed in Table 1.

Size Exclusion and Light Scattering—Light scattering (LS) data were collected by size exclusion chromatography using a Superdex 200, 10/300, HR column (GE Healthcare) that was connected to an Agilent 1200 high performance liquid chromatography (HPLC) system (Agilent Technologies, Wilmington, DE) equipped with an autosampler. Elution was monitored using a photodiode array UV-visible detector (Agilent Technologies), a differential refractometer (OptiLab rEx, Wyatt Technology Corp., Santa Barbara, CA) and static and dynamic, multilangle laser LS detector (HELEOS II with Quasi-Elastic-Light-Scattering capability, Wyatt Technology). The size exclusion chromatography-UV/LS/refractive index system was equilibrated in 20 mM Tris (pH 8) and 150 mM NaCl, with a flow rate of 0.5 ml/min. For data collection and analysis, two software packages were used. The ChemStation software (Agilent Technologies) controlled the operation of the HPLC and data collection from the UV-visible detector, whereas the ASTRA software (Wyatt Technology) was used for data collection from the refractive index and LS detectors and to record


**α-Catenin-Vinculin Complex Crystal Structure**

the 280-nm UV trace from the photodiode array detector. ASTRA software was used to determine the weight average molecular mass across the entire elution profile in 1-s intervals from static LS measurements as described (36).

**Native Gel and F-actin Co-sedimentation Assays**—All binding studies were performed in 20 mM Tris-HCl (pH 8), 150 mM NaCl, and 5 mM DTT. VH-Vt or VH-α-catenin (82–906, 82–634, or 263–634) complexes were obtained by incubating equimolar amounts (15 μM) of proteins for 10 min at room temperature. Complexes were then incubated with Vt or α-catenin, respectively, at equimolar and 5-fold excess concentration for an additional 10 min. The samples were analyzed by PhastGel using native PhastGel buffer strips (homogeneous 20% for Fig. 4, C–E or 10–15% gradient for Figs. 4, A and B, and 5) and stained with Coomassie Blue. Similarly, VBS (residues 332–353) was added to preformed Vh1-Vt complexes at 5-fold excess concentration. F-actin pulldown assays were carried out as described (37).

**RESULTS**

Structured Unfurled VBD of α-Catenin in Complex with Vh1—Binding studies have suggested that α-catenin residues 327–402 were necessary for binding of α-catenin to vinculin (38, 39), and more recently, the minimal VBS was determined as residues 325–360 (40) or 326–377 (41). We determined the α-catenin VBD as residues 277–382 based on secondary structure analyses and homology model prediction (42) and co-crystallized the Vh1-VBD complex. The crystals belong to the orthorhombic space group P2₁2₁2₁ with unit cell dimensions of a = 57.2 Å, b = 73.9 Å, and c = 107.4 Å (Table 1) with one heterodimer in the asymmetric unit (Z = 4) resulting in a solvent content of 59% and a crystal volume per unit of protein molecular weight, Vₘ (43), of 3.03 Å³/Da. The Vh1-VBD structure was determined to 2.7 Å resolution. The regions corresponding to Vh1 residues 219–222 and α-catenin 277–289 were not modeled due to lack of discernible electron density, and although the electron density for the remainder of the α-catenin residues 354–361 is weak, the connectivity is unambiguous (supplemental Fig. S2 where the two four-helix bundles (4HB) subdomains are connected by one centrally shared α-helix (α4) (Fig. 1A). As first reported for Vh1 bound to talin-VBS3 (22), the minimal α-catenin VBS (residues 328–352) forms an α-helix that binds the N-terminal 4HB of Vh1 by helix bundle conversion, as subsequently also seen for the VBSs of talin (22, 28, 45–48), α-actinin (49), and the bacterial IpaA (32, 50, 51) and scad4 (52) in complex with Vh1. Interestingly, although α-catenin residues 328DRRERIYAECNAVQRALQDLS³⁴⁹ align with talin VBS3 residues E1945ELIECARRVEKSVHVLALQA¹⁹⁷⁰ (41), talin residues E1945ELIESARKVSEKSVHLALQA¹⁹⁶⁹ (Protein Data Bank (PDB) entry 1rkc) are structurally equivalent to α-catenin residues R³²⁸RIVAECNAVQRALQDLSG³³⁵ i.e. shifted in register by 4 residues.

**TABLE 1**

| X-ray data reduction statistics | Space group | P2₁,2,2 |
|--------------------------------|-------------|---------|
| Resolution (last shell)        | 107.4 Å – 2.65 Å (2.8 Å – 2.65 Å) |
| Total measurements             | 98,915 |
| Number of unique reflections   | 13,785 (1.970) |
| Wavelength                     | 1 Å |
| R-merge¹ (last shell)          | 0.068 (0.485) |
| Average I/σ(I) (last shell)    | 21.2 (3.8) |
| Completeness (last shell)      | 1 (1) |
| Redundancy (last shell)        | 7.2 (7.3) |

**Crystallographic refinement statistics**

| Resolution (last shell) | 50 Å – 2.66 Å (2.87 Å – 2.66 Å) |
| No. of reflections (working set) | 12,951 |
| No. of reflections (test set) | 686 |
| R-factor² (last shell) | 0.193 (0.202) |
| No. of amino acid residues | 341 |
| No. of protein atoms | 2,678 |
| No. of solvent molecules | 82 |
| No. of acetate atoms | 36 |
| Average B-factor |
| Protein | 64.8 Å² |
| Solvent | 56.6 Å² |
| Acetate | 70.4 Å² |
| r.m.s.d.³ from ideal geometry |
| Bond lengths | 0.01 Å |
| Bond angles | 1.06° |
| Ramachandran plot statistics |
| Most favored | 98.8% |
| Additional allowed | 1.2% |
| Generously allowed | 0.0% |
| Disallowed | 0.0% |

| F(obs) | F(calc) |
|--------|---------|
| Rmerge = \frac{\sum_{hkl} \left| F_{obs}(hkl) \right| - \left| F_{calc}(hkl) \right|}{\sum_{hkl} \left| F_{obs}(hkl) \right|} |
| R-factor = \frac{\sum_{hkl} \left| F_{calc}(hkl) \right| - \left| F_{obs}(hkl) \right|}{\sum_{hkl} \left| F_{calc}(hkl) \right|} |

* R-merge is a cross-validation residual calculated by using about 5% reflections, which were randomly chosen and excluded from the refinement.

**FIGURE 1. α-Catenin is unfurled when bound to vinculin.** A, the crystal structure of Vh1 (residues 1–258; yellow) in complex with α-catenin VBD shows an extended conformation of α-catenin with extensive interactions with both subdomains of Vh1. Vh1 α-helices and α-catenin termini are labeled. Residues 305–316 are shown in blue, 328–351 are in magenta, and the remainder of the α-catenin residues are in cyan. For clarity, residues 290–304 and 362–382 are not shown. B, surface representation showing the respective interfaces (white, carbon; red, oxygen; blue, nitrogen; yellow, sulfur). Vh1 is in the same orientation as shown in A, and α-catenin is rotated 180° down with respect to its orientation shown in A.

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Unexpectedly, a second α-helix (α-catenin residues 305–316) binds the C-terminal 4HB of Vh1 in a novel fashion, by packing its hydrophobic face against the first (α4) and second (α5) α-helices of the C-terminal Vh1 4HB (Fig. 1A). Collectively, these dual interactions create a large hydrophobic interface that buries nearly 25% of the VBD solvent-accessible surface area (over 2,300 Å²) and about 14% of that present on Vh1 (over 1,900 Å²). These interactions include 12 hydrogen bonds (vinculin residues Thr-8 with α-catenin Arg-330, Pro-15 with α-catenin Lys-358, Gln-19 with α-catenin Gln-345 and Ser-349, His-27 with α-catenin Tyr-351, Thr-61 and Thr-64 with α-catenin Arg-329, Arg-136 with α-catenin Gly-315, and Lys-170 with α-catenin Ala-317 and Met-319) and novel hydrophobic interactions (vinculin residues Val-137, Gly-140, Ile-141, and Gly-167 with α-catenin Leu-318) (Fig. 1B, supplemental Fig. S3A). In comparison, the buried solvent-accessible surface area in other Vh1-VBS structures are only about 1,200 Å² (22).

Although two IpaA-VBSs have been shown to simultaneously bind to Vh1, where the second binding site is located between two different α-helices (α5 and α6) in the C-terminal 4HB (supplemental Fig. S2B), these were short IpaA-VBS peptides (22 residues) that bind with 2:1 molar ratio (32) and that bury less than 1,300 Å² of solvent-accessible surface area. Further, no hydrogen bonds were observed for this interaction (32). In contrast, our Vh1-VBD structure is the first report of an entire helix bundle (116 residues) having good electron density that connects two individual α-helices that bind to vinculin. Importantly, in our Vh1-VBD structure, the binding sites for α-catenin on both subdomains of Vh1 are located on the surface of full-length vinculin. Thus, these regions are accessible for α-catenin binding to the full-length vinculin in its inactive, closed conformation (supplemental Fig. S4).

Two additional α-helices of the α-catenin VBD seen in our crystal structure, one on each terminus, engage in further and novel interactions with vinculin (supplemental Fig. S5). Although the N-terminal α-helix is quite short (residues 293–300) (supplemental Fig. S4) and makes crystal contacts with α-helices α5 and α6 of a symmetry-related Vh1 molecule (supplemental Figs. S3B and S5B), the C-terminal α-helix lies almost parallel (at about 15°) to a helix α2 from a two-fold related Vh1 molecule (Fig. 2B, supplemental Fig. S4). The resulting dimer seems further stabilized by a disulfide bond that is formed between Cys-324 and its symmetry-related mate (Fig. 2, A and C). Although α-catenin is predominantly monomeric but also found as a dimer in solution (53), we found that our Vh1-VBD complex, as well as unbound VBD, is either monomeric or dimeric in solution (Fig. 3). Furthermore, the elution of VBD is consistent with a compact globular domain in solution. Thus, the VBD unfurls upon binding to vinculin in accord with calorimetry data showing that α-catenin (residues 273–510) bound to the VH domain (residues 1–718) with nm affinity and that the binding was endothermic and entropy-driven, consistent with a major conformational change (39). Collectively, our data are consistent with the notion that α-catenin unfurls to bind to vinculin, allowing dimerization.

**Vinculin-α-Catenin Binding Is Facilitated by Severing Vinculin Head-Tail Interaction**—To avoid potential nonspecific effects on the binding, we assessed the binding properties of untagged α-catenin and vinculin proteins. Because deletion of residues 1–81 increases the stability of α-catenin (20), we thus cloned α-catenin residues 82–906 (hereafter referred to as full-length) for our binding studies. α-Catenin was reported to bind weakly to full-length vinculin (16, 39). Indeed, only a fraction of full-length α-catenin and vinculin formed a complex in solution, as seen in our native gel shift assays (Fig. 4A). Thus, full-length α-catenin in its inactive conformation is not sufficient to activate vinculin. However, the minimal α-catenin-VBS is sufficient for severing the vinculin Vh1-Vt interaction to form a

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**FIGURE 2. α-Catenin-vinculin complex crystal structure.** Vh1 is shown in cyan or green, and its respective bound α-catenin is shown in white or magenta. A, α-catenin Cys-324 forms a disulfide bond with a two-fold related molecule. Likewise, the C-terminal α-catenin α-helix binds to the second Vh1 α-helix of a two-fold related heterodimer burying over 500 Å² of solvent-accessible surface area with a shape correlation statistic derived using the CCP4 program SC (55) of over 0.8 for this interface, a significant value where a value of 1 indicates a perfect fit versus 0.35, which indicates the mismatch of an artificial association. Vh1 α-helices are labeled, as well as some α-catenin residues 324 and 373. B, close-up view of the dimer interface. Vinculin residues are shown in cyan, and α-catenin residues are in magenta as in A and in a slightly rotated view from A. C, space-filling representation of the dimer of heterodimers (same color coding as shown in A) and rotated about 180° about the horizontal axis of the A image. Some contact residues are indicated.
novel Vh1-VBS complex (Fig. 4B), as seen for the VBSs of talin (22, 28, 45–48), α-actinin (49), or the bacterial IpaA (32, 50, 51) and sca4 (52).

Co-localization studies of α-catenin proteins have suggested that an inhibitory domain (α-catenin residues 510–697) prevents vinculin binding (40). However, full-length α-catenin harboring the 510–697 region readily bound to VH (the globular head domain of vinculin, residues 1–843) (Fig. 4C). Surprisingly, the Vt efficiently displaced full-length α-catenin (Fig. 4C) and more truncated versions of α-catenin (residues 82–634, Fig. 4D; or residues 263–634, Fig. 4E) from preformed VH-α-catenin complexes. Thus, intramolecular interactions of vinculin are preferred over those with α-catenin.

F-actin Binds α-Catenin-Vinculin Complex via the C Termi- nus of Either α-Catenin or Vinculin—The binding of full-length vinculin to F-actin has been reported to be enhanced by α-catenin (residues 273–510) (54). These findings, together with Vt displacement of α-catenin from α-catenin-VH complexes, suggested that once bound to F-actin, Vt cannot displace α-catenin. To test this notion, F-actin co-sedimentation studies were performed. The vinculin VH domain (residues 1–840) formed a ternary complex with α-catenin (residues 82–906, lane 2; complex, α-HV, lane 3) that migrates farther than Vh1 alone (lane 1). C–E, reciprocal competition assays by titrating equimolar concentrations (15 μM) or 5-fold excess concentrations (75 μM) of α-catenin (lanes 3 and 4) residues 82–906 (C), residues 82–634 (D), or 263–634 (E) to VH-Vt complex as analyzed by native gel shift mobility assays. VH alone (lane 1), VH-Vt (lane 2), VH-α-catenin (lane 3), and α-catenin (lane 4) are also shown. As is evident, all three α-catenin constructs fail to displace Vt from preformed VH-Vt complexes (lanes 3 and 4). In contrast, Vt readily displaces all three α-catenin constructs from preformed VH-α-catenin complexes (lanes 6 and 7). VH, vinculin head domain (residues 1–843); Vt, vinculin tail domain (residues 879–1066).
interaction by binding to the C terminus of α-catenin and/or vinculin.

DISCUSSION

Stabilization of AJs to the actin cytoskeleton is necessary for maintenance of cell-cell contacts (17), yet the mechanism by which this occurs is not resolved. One clear contributor to the response is α-catenin, which is necessary for the formation and stabilization of AJs, for recruitment of vinculin to these sites, and for linking cadherin complexes to the actin cytoskeleton (16–19). α-Catenin also controls branched actin polymerization (53) and directly associates with F-actin during junction reinforcement (40). Our findings suggest that a complex interplay of vinculin, α-catenin, and the F-actin network are together necessary to stabilize AJs.

The structure of the vinculin Vh1-α-catenin VBD complex shows that α-catenin unfurls upon complex formation, allowing dimerization (Fig. 2), and that it has a novel vinculin binding mode, where two α-helices in the VBD bind simultaneously to the four-helix bundle subdomains of the Vh1 domain. Further, the α-catenin binding sites are fully accessible in full-length vinculin (supplemental Fig. S4), and the entire globular head VH domain readily binds to α-catenin (Fig. 4, C–E). However, the two full-length proteins interact only weakly in solution (Fig. 4A), and competitive binding studies support a model whereby vinculin in its open conformation binds to F-actin and then binds to and unfurls α-catenin to form a stable ternary complex.

Vinculin recruitment by α-catenin is force-dependent (40). However, the intramolecular head-tail interaction of vinculin impairs α-catenin-vinculin interaction. Furthermore, the formation and/or stabilization of the α-catenin-vinculin complex is greatly stabilized by the binding of either partner to F-actin through their C-terminal domains. Here proximity rules may come into play, where F-actin-bound α-catenin promotes the association of vinculin with F-actin as vinculin is a highly dynamic protein and exists in equilibrium between its closed and open states. Collectively, these findings suggest that cytoskeletal tension stabilizes the α-catenin-vinculin complex at AJs.

α-Catenin deletion mutants that retain the VBD but cannot bind to the cytoskeleton can recruit vinculin to AJs, which led to the hypothesis that a region C-terminal of the VBD prevents α-catenin binding to vinculin and that this inhibition is relieved when α-catenin is bound to the actin cytoskeleton (40). Furthermore, binding studies with truncated proteins suggested the hypothesis that α-catenin activates vinculin (41). While α-catenin binds vinculin weakly in native gel assays, our competition binding assays show that α-catenin readily binds to VH, yet is displaced by Vt. Thus, the nanomolar affinity of the intramolecular VH-Vt interaction prevents α-catenin binding and vinculin needs to be in its open state to bind to α-catenin. Collectively, the data indicate that α-catenin does not activate vinculin but rather that F-actin bound-vinculin unfurls and binds to α-catenin to stabilize AJs.

What is still not resolved is what trigger activates vinculin at AJs. One possibility is that this response simply relies on an equilibrium of vinculin in its open versus activated state, where

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**FIGURE 5. Vinculin-α-catenin complex co-sediments with F-actin via F-actin binding domain (FABD) of either α-catenin or vinculin. A, the α-catenin-VH complex binds F-actin via the α-catenin FABD. About 30 nM of either vinculin or α-catenin was used in the corresponding reaction mixtures.**

Left gel, lanes 1 and 2, F-actin is found in the pellet (P). Lanes 3 and 4, α-catenin does not aggregate and remains in the soluble fraction (S). Lanes 5 and 6, α-catenin binds to F-actin. Right gel, lanes 1 and 2, the αH-α-catenin complex does not aggregate. Lanes 3 and 4, the αVH-α-catenin complex binds to F-actin via the α-catenin FABD. Lanes 5 and 6, VH does not bind to F-actin. Molecular weight markers are shown for the left gel.

**B**

Vh1-Vt

α-catenin

α-cateninΔFABD

α-catenin 263–634

α-catenin-Vh1-Vt

α-cateninΔFABD-Vh1-Vt

α-catenin 263–634

**C**

α-cat

F-act

VH

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S

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REFERENCES

1. Braga, V. M., and Balda, M. S. (2004) Regulation of cell-cell adhesion. Semin. Cell Dev. Biol. 15, 631–632
2. Pokutta, S., and Weis, W. I. (2002) The cytoplasmic face of cell contact sites. Curr. Opin. Struct. Biol. 12, 255–262
3. Clevers, H. (2006) Wnt/β-Catenin signaling in development and disease. Cell 127, 469–480
4. Wahl, J. K., 3rd, Kim, Y. J., Cullen, J. M., Johnson, K. R., and Wheelock, M. J. (1998) Vinculin is part of the cadherin-catenin junctional complex: the latter can bind to F-actin that then facilitates interactions with α-catenin. Alternatively, vinculin may bind to other partners at AJs to augment this response, for example, to the Arp2/3 complex, as Arp2/3-directed actin polymerization is suppressed by α-catenin (11).
5. Drees, F., Pokutta, S., Yamada, S., Nelson, W. J., and Weis, W. I. (2005) Deconstructing the cadherin-catenin-actin complex. Cell 123, 899–901
6. Weis, W. I., and Nelson, W. J. (2006) Re-solving the cadherin-catenin-actin conundrum. J. Biol. Chem. 281, 35593–35597
7. Weis, W. I., and Nelson, W. J. (2005) Reconstructing the cadherin-catenin-actin complex. Cell 123, 899–901
8. Boggs, P. R., O’Hara, B. P., Nietlispach, D., Kirkpatrick, J., and Izard, T. (2006) The vinculin binding sites of talin and α-actin are sufficient to activate vinculin. J. Biol. Chem. 281, 7228–7236
9. Bois, P. R., O’Hara, B. P., Nietlispach, D., Kirkpatrick, J., and Izard, T. (2006) The vinculin binding sites of talin and α-actin are sufficient to activate vinculin. J. Biol. Chem. 281, 7228–7236
10. Miyamura, Y., Isho, M., Maeno, Y., Tsukita, S., and Nagauchi, A. (1999) Functional domains of α-catenin required for the strong state of cadherin-based cell adhesion. J. Cell Biol. 144, 1311–1322
42. Kelley, L. A., and Sternberg, M. J. (2009) Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. 4, 363–371
43. Matthews, B. W. (1968) Solvent content of protein crystals. J. Mol. Biol. 33, 491–497
44. Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D. Biol. Crystallogr. 66, 12–21
45. Izard, T., and Vonrhein, C. (2004) Structural basis for amplifying vinculin activation by talin. J. Biol. Chem. 279, 27667–27678
46. Gingras, A. R., Ziegler, W. H., Frank, R., Barsukov, I. L., Roberts, G. C., Critchley, D. R., and Emsley, J. (2005) Mapping and consensus sequence identification for multiple vinculin binding sites within the talin rod. J. Biol. Chem. 280, 37217–37224
47. Fillingham, I., Gingras, A. R., Papagriforou, E., Patel, B., Emsley, J., Critchley, D. R., Roberts, G. C., and Barsukov, I. L. (2005) A vinculin binding domain from the talin rod unfolds to form a complex with the vinculin head. Structure 13, 65–74
48. Yogesha, S. D., Rangarajan, E. S., Vonrhein, C., Bricogne, G., and Izard, T. (2012) Crystal structure of vinculin in complex with vinculin binding site 50 (VBS50), the integrin binding site 2 (IBS2) of talin. Protein Sci. 21, 583–588
49. Bois, P. R., Borgon, R. A., Vonrhein, C., and Izard, T. (2005) Structural dynamics of α-actin-vinculin interactions. Mol. Cell. Biol. 25, 6112–6122
50. Izard, T., Tran Van Nhieu, G., and Bois, P. R. (2006) Shigella applies molecular mimicry to subvert vinculin and invade host cells. J. Cell Biol. 175, 465–475
51. Park, H., Valencia-Gallardo, C., Sharff, A., Tran Van Nhieu, G., and Izard, T. (2011) Novel vinculin binding site of the IpaA invasin of Shigella. J. Biol. Chem. 286, 32214–32221
52. Park, H., Lee, J. H., Gouin, E., Cossart, P., and Izard, T. (2011) The Rickettsia surface cell antigen 4 applies mimicry to bind to and activate vinculin. J. Biol. Chem. 286, 35096–35103
53. Benjamin, J. M., Kwiatkowski, A. V., Yang, C., Korobova, F., Pokutta, S., Svitkina, T., Weis, W. L., and Nelson, W. J. (2010) αE-Catenin regulates actin dynamics independently of cadherin-mediated cell-cell adhesion. J. Cell Biol. 189, 339–352
54. Janssen, M. E., Kim, E., Liu, H., Fujimoto, L. M., Bobkov, A., Volkmann, N., and Hanein, D. (2006) Three-dimensional structure of vinculin bound to actin filaments. Mol. Cell 21, 271–281
55. Lawrence, M. C., and Colman, P. M. (1993) Shape complementarity at protein/protein interfaces. J. Mol. Biol. 234, 946–950
56. Cohen, D. M., Chen, H., Johnson, R. P., Choudhury, B., and Craig, S. W. (2005) Two distinct head-tail interfaces cooperate to suppress activation of vinculin by talin. J. Biol. Chem. 280, 17109–17117