Published in final edited form as:

Biochem Soc Trans. 2008 April ; 36(Pt 2): 141–147. doi:10.1042/BST0360141.

Biochemical and structural analysis of α -catenin in cell–cell contacts

Sabine Pokutta^{*,†}, Frauke Drees^{†,‡}, Soichiro Yamada^{†,‡}, W. James Nelson^{†,‡}, and William I. Weis^{*,†,1}

^{*}Department of Structural Biology, D100 Fairchild Science Building, Stanford University School of Medicine, Stanford, CA 94305-5126, U.S.A

[†]Department of Molecular and Cellular Physiology, Room B100, Beckman Center, Stanford University School of Medicine, 279 Campus Drive, Stanford, CA 94305-5345, U.S.A.

[‡]Department of Biological Sciences, Gilbert Hall, Stanford University, Stanford, CA 94305-5020, U.S.A

Abstract

Cadherins are transmembrane adhesion molecules that mediate homotypic cell–cell contact. In adherens junctions, the cytoplasmic domain of cadherins is functionally linked to the actin cytoskeleton through a series of proteins known as catenins. E-cadherin binds to β -catenin, which in turn binds to α -catenin to form a ternary complex. α -Catenin also binds to actin, and it was assumed previously that α -catenin links the cadherin–catenin complex to actin. However, biochemical, structural and live-cell imaging studies of the cadherin–catenin complex and its interaction with actin show that binding of β -catenin to α -catenin prevents the latter from binding to actin. Biochemical and structural data indicate that α -catenin acts as an allosteric protein whose conformation and activity changes depending on whether or not it is bound to β -catenin. Initial contacts between cells occur on dynamic lamellipodia formed by polymerization of branched actin networks, a process controlled by the Arp2/3 (actin-related protein 2/3) complex. α -Catenin can suppress the activity of Arp2/3 by competing for actin filaments. These findings lead to a model for adherens junction formation in which clustering of the cadherin– β -catenin complex recruits high levels of α -catenin that can suppress the Arp2/3 complex, leading to cessation of lamellipodial movement and formation of a stable contact. Thus α -catenin appears to play a central role in cell–cell contact formation.

Keywords

actin; actin-related protein 2/3 complex (Arp2/3 complex); adherens junction; cadherin; catenin; cell adhesion

Introduction

Cadherins are calcium-dependent cell adhesion molecules whose extracellular regions mediate homotypic cell–cell adhesion. The classical cadherins are found in adherens junctions, cell–cell contact structures that are associated with the actin cytoskeleton [1,2]. The co-ordination of adhesion with the underlying cytoskeleton represents an essential part of cell and tissue morphogenesis. For example, the invagination of an epithelial sheet to

form a tube requires changes in the shapes and tensile properties of the constituent cells while the cells remain attached to one another [3].

Cell adhesion and changes in the cytoskeleton are also co-ordinated during the initial formation of cell–cell contacts [4,5]. The surface of non-contacting epithelial cells features highly dynamic lamellipodia driven by the polymerization of branched actin networks underneath the membrane [6]. Initial contacts between cells are made by small portions of their lamellipodia, which bear cadherins on their surfaces. As the contact area enlarges, cadherins are observed to concentrate in the contacting region, and the lamellipodial dynamics of the apposed membranes are reduced as a stable contact forms [7]. Thus there is an observable correlation between the formation of a cadherin-based contact and the cessation of membrane-protrusive activity driven by actin polymerization. Moreover, in a mature contact, the branched actin networks associated with lamellipodia are replaced with bundles of actin cables that abut the membrane [8].

The importance of cell adhesion in tissue formation and wound healing, and its dysregulation in many diseases, makes understanding the molecular basis of cell-contact formation and the structure of the resulting contacts of great importance. Models of the molecular organization of adherens junctions initially came from the discovery of the catenins. Immunoprecipitation of cadherins from detergent-extracted epithelial cells led to the discovery of β - and α -catenin [9]. β -Catenin binds tightly to the highly conserved cytoplasmic portion of classical cadherins, with $K_d = 30$ nM–52 pM depending on the phosphorylation state of E-cadherin [10]. β -Catenin also binds to α -catenin [11], albeit more weakly (see below). α -Catenin binds to β -catenin [11,12] and to F-actin [13,14]. These protein–protein interactions were demonstrated pairwise, i.e. cadherin binds to β -catenin, β -catenin binds to α -catenin, and α -catenin binds to actin. The ternary complex of E-cadherin, β -catenin and α -catenin is also readily apparent in immunoprecipitation and reconstitution experiments [11]. On the basis of these findings, a minimal model of junction organization is that cadherins are linked directly to actin through the catenins (Figure 1).

In MDCK (Madin–Darby canine kidney) cells, immunofluorescence studies have shown that β -catenin binds to cadherin while still in the endoplasmic reticulum, and the two proteins move together to the cell surface [15]. Disruption of this interaction results in proteasomal destruction of cadherin [16]. This probably reflects the fact that the cadherin cytoplasmic domain is intrinsically unstructured and contains a PEST (Pro-Glu-Ser-Thr) motif associated with recognition by the ubiquitination machinery [17]. The crystal structure of the cadherin– β -catenin complex shows that this sequence contacts β -catenin in the complex, and is probably protected from ubiquitin ligase recognition by β -catenin [18]. Thus the cadherin– β -catenin complex can be considered a single unit that traffics to the cell surface.

α -Catenin is a 906-amino-acid protein that contains three regions with strong homology with the actin-binding protein vinculin (Figure 2). A number of α -catenin-binding partners have been reported, but we focus here on β -catenin, which binds to a region near the N-terminus (residues 57–146) [12], and F-actin, which binds to the C-terminal region (residues 697–906) [13]. In addition, epithelial α -catenin (α -E-catenin) binds to itself, using a domain that overlaps the β -catenin-binding site. This domain, defined by limited proteolysis as residues 82–264, consists of five α -helices [13] (Figure 3). The domain starts as a 30-residue helix, and is followed by a 50-residue helix, the first half of which interacts in an antiparallel manner with the first helix. The second half of the long helix is part of the four-helix bundle that forms the C-terminal end of the domain. The homodimer forms by association of the two N-terminal helices, creating a non-covalent four-helix bundle (Figure 3).

Mutagenesis studies have shown that amino acids 117–149 of β -catenin comprise the α -catenin-binding site [19]. This sequence was predicted to form an amphipathic helix, and replacement of residues on the hydrophobic face of the helix diminished binding to α -catenin. The β -catenin-binding site of α -catenin included residues 57–146 [12]. Although residues 57–81 were removed by proteolysis and were not part of the crystallized dimerization domain, these residues also are predicted to form an amphipathic helix. β - and α -catenin interact as a 1:1 heterodimer, indicating that binding of β -catenin competes with homodimerization of α -catenin. On the basis of these observations, we proposed that, when β -catenin binds to α -catenin, α -catenin residues 57–81, which are otherwise flexibly linked to the dimerization domain that starts at residue 82, bind to the unpaired helices at the N-terminus of the dimerization domain, and β -catenin 117–149 forms the fourth helix of a new four-helix bundle [12] (Figure 3). The competition between the α -catenin homodimer and the β -catenin heterodimer explains the relatively weak interaction between these two proteins.

To overcome the competition with homodimerization and examine the β -catenin-binding interface structurally, we used a chimaera approach in which the α -catenin-binding region of β -catenin, residues 118–151, were fused with a polyglycine linker to the N-terminus of α -catenin 57–264 [12]. The structure of this fusion protein, designated $\beta\alpha$ -catenin, revealed that the β -catenin sequence from 118 to 146 forms the expected amphipathic helix, and a four-helix bundle that includes α -catenin 57–81 is observed. The packing of these helices on to the other α -catenin helices differs slightly from that observed in the homodimer, however.

The actin-binding activity of α -catenin resides in the C-terminal region, which bears 34% sequence identity with the actin-binding region of vinculin. Although there is no crystal structure of the domain from α -catenin, the domain from vinculin is a five-helix bundle [20]. On the basis of standard actin co-sedimentation assays, the affinity of the α -catenin–F-actin interaction has been estimated to be 0.3 μ M [14].

Can α -catenin link the cadherin– β -catenin complex to actin?

To understand further the biochemistry and structure of the junctional assembly, we attempted to reconstitute the cadherin–catenin–actin complex *in vitro*. Recombinantly expressed and purified E-cadherin cytoplasmic domain, β -catenin and α -catenin were mixed with F-actin at sufficiently high concentrations to form the quaternary complex based on the known or estimated affinity constants, then centrifuged to sediment the actin and any stably bound proteins. A portion of the input α -catenin was observed to sediment with actin, as expected, but none of the cadherin or β -catenin co-sedimented with α -catenin above background levels [21]. Moreover, as the input concentration of the cadherin– β -catenin complex was increased, less α -catenin co-sedimented, suggesting that binding of α -catenin to β -catenin and actin is mutually exclusive. This was confirmed by preparing a new $\beta\alpha$ -catenin chimaera in which the α -catenin-binding region of β -catenin was fused to residues 57–906 of α -catenin, i.e. extending through the C-terminal actin-binding domain of α -catenin. This chimaera did not bind to F-actin significantly, confirming that binding to β -catenin prevents α -catenin from binding to F-actin [21].

The results outlined above demonstrate that α -catenin cannot link β -catenin to actin as assumed in the standard models of adherens junctions. Since these experiments use highly purified recombinant proteins, a concern is that other factors present in native junctional membranes or cytosol might be required to establish a connection to actin. Several approaches were taken to address this problem. To examine whether native membranes are needed, for example cadherin clustering or lipids, patches of membranes containing junctional complexes were prepared [21]. This was accomplished by depositing cells on

microscope slides on which the extracellular domain of E-cadherin is attached. In this way, the surface of the cell closest to the slide can form cadherin-based contacts. The cells are then ruptured by brief sonication. After washing away the released cell contents, a patch of membrane containing cadherins and their associated cytosolic proteins remains on the slide. Immunofluorescence confirmed that the patches contain the cadherins, catenins and actin. We then exploited the fact that the cadherin cytoplasmic domain is natively unstructured, and can therefore withstand denaturing conditions. Treatment of the patches with guanidine removes the actin and catenins. Recombinant catenins could then be added back to these patches, showing that the cadherin–catenin complex can be restored. However, as observed in the solution experiments, F-actin did not bind to the reconstituted cadherin–catenin complex on the patches [21]. Moreover, addition of either vinculin or α -actinin, both of which are reported to bind to α -catenin, did not restore actin binding, nor did addition of cytosol, indicating that soluble factors are not responsible for actin binding [21].

Despite these results, there are still concerns that the inability to observe linkage between α -catenin and actin could be due to missing components that were not tested. It is important to note, however, that immunoprecipitation of cadherins only brings down stoichiometric amounts of catenins and not other proteins. Although it could be argued that a missing factor might have been attached to actin, it would seem most likely that a required structural component of the junction would be present at levels comparable with the catenins in these experiments. Another concern when using recombinant proteins is the lack of post-translational modifications that could affect binding affinities. Although the cytosol add-back experiments partly address this issue, we also examined whether casein kinase II, which has been reported to phosphorylate α -catenin, might affect the interactions of α -catenin. However, *in vitro* phosphorylation of α -catenin by this kinase did not affect the outcomes of the co-sedimentation experiments [21].

The biochemical data presented above argue strongly against a stable linkage between the cadherin–catenin complex and actin. Nonetheless, it is essential to ask whether the results obtained *in vitro* with purified recombinant proteins correlate with *in vivo* behaviour. Using GFP (green fluorescent protein)-tagged versions of cadherins, catenins and actin, we carried out FRAP (fluorescence recovery after photobleaching) experiments to examine the dynamics of these proteins at the junctional membrane [21]. In particular, if the cadherin–catenin complex is linked stably to the actin cytoskeleton, we would expect to see similar diffusional behaviours and mobilities of these proteins on the membrane. We examined two parameters from FRAP measurements: the halftime of fluorescence recovery $t_{1/2}$, which occurs at a rate dictated by interactions that constrain free diffusion, and the fraction of the fluorescence intensity that recovers, which defines the mobile fraction of molecules.

GFP-tagged E-cadherin, β -catenin and α -catenin (all expressed at a fraction of the endogenous level to avoid over-expression artefacts) all displayed similar $t_{1/2}$ and mobile fraction values [21] (Figure 4). This was also true if the cytoplasmic domain of cadherin was deleted, thereby breaking all potential connection to the cytoskeleton, or if the actin-binding domain of α -catenin was deleted to disrupt the potential connection to actin. In contrast, actin near the membrane showed a much shorter $t_{1/2}$ and a larger mobile fraction than the cadherin–catenin complex (Figure 4). Thus the FRAP data indicate that the cadherin–catenin complex is not linked stably to the underlying actin cytoskeleton in confluent MDCK cells [21]. Moreover, FLIP (fluorescence loss in photobleaching) experiments, in which a region of cytosol is bleached and the loss of fluorescence from the contact site monitored as the fluorescent pool exchanges with the cytosol, showed that the actin at cell–cell contacts is unusually dynamic compared with that associated with cell–substratum adhesions [21].

What is the role of α -catenin in cell–cell contacts?

Although the biochemical, membrane patch and live-cell imaging data indicate a lack of stable linkage between the cadherin–catenin complex and the actin cytoskeleton, it is well known that actin associates with cell–cell contacts and that actin dynamics are important for the induction of cell–cell adhesion. Moreover, it was noted in the Introduction that cadherin-mediated cell–cell adhesion induces changes in actin organization and dynamics at cell–contact sites. We therefore asked whether α -catenin might have a role in this process.

It was noted above that recombinant α -E-catenin is largely dimeric when purified. To assess the state of α -catenin in the cell, we subjected extracts of MDCK cells to gel-filtration chromatography using recombinant monomeric and dimeric α -catenin as size standards. Most of the extracted α -catenin proved to be monomeric. The dimeric fraction was a mixture of α -catenin homodimer and α - β -catenin heterodimer. Since our earlier studies had shown that homodimerization competes with β -catenin binding, we would expect that the monomeric α -catenin would bind more strongly to β -catenin. This was confirmed by gel-filtration experiments mixing β -catenin with either monomeric or dimeric α -catenin. This gave an approximate K_d value of 1 μ M for the interaction of monomeric α -catenin with β -catenin [22].

We next examined the actin-binding activities of monomeric and dimeric α -catenin [22]. As would be expected from a simple valency argument, dimeric α -catenin binds to actin better than monomeric α -catenin. However, because it is impossible to prepare 100% pure monomeric α -catenin, it is possible that the observed actin binding by α -catenin monomer is due to contaminating dimer. We attempted to probe the molecular conformation of α -catenin in its monomer, homodimer and β -catenin heterodimer states using limited proteolysis. The proteolytic digestion patterns were different in each case, suggesting that distinct molecular conformations are associated with these states [22]. The different binding activities, particularly the inhibition of actin binding by β -catenin, as well as the proteolytic sensitivity data, suggest that α -catenin is an allosteric protein in which the binding of one ligand alters its conformation and changes its affinity for another ligand.

The mutually exclusive binding to β -catenin or actin implies that α -catenin must dissociate from the cadherin– β -catenin complex before it can bind actin. This was confirmed by purifying the cadherin– β -catenin– α -catenin complex and incubating it with F-actin at various concentrations or times before sedimentation. Approx. 20% of α -catenin dissociates from 5 μ M pre-formed complex [22]. FLIP experiments confirmed that, in living cells, membrane-associated α -catenin can exchange with the cytosolic pool [22].

The ability of α -catenin to associate with the cadherin– β -catenin complex at cell–cell contacts, but also to dissociate and bind actin, led us to examine whether it might have a role in the changes in actin organization that accompany cell–contact formation. As noted in the Introduction, lamellipodial activity decays as the cadherin–catenin complex accumulates and the contact matures [4,7,23]; as the contact forms, the actin cytoskeleton reorganizes from the branched polymer that drives lamellipodia formation to a linear cable that lies under the membrane [8]. Formation of the branched actin network is mediated by the Arp2/3 (actin-related protein 2/3) complex, which binds to the sides of actin filaments and promotes growth of a new filament at a 70° angle with respect to the first [6]. Lamellipodial movements generated by Arp2/3-mediated polymerization of branched actin networks result in transient cell–cell contacts between overlapping lamellipodia, and lack of Arp2/3 activity blocks formation of cell–cell contacts [24].

Cells from α -catenin-knockout mice lose contact inhibition and display increased migratory activity [25,26]. Furthermore, decreased levels of α -catenin result in increased membrane

activity in neurons, whereas overexpression of α -catenin suppresses membrane activity [27]. Given these data, we asked whether α -catenin might influence Arp2/3-mediated branched actin polymerization and regulate membrane protrusive activity. Using a standard fluorescent actin polymerization assay, we found that α -catenin can suppress Arp2/3-mediated actin polymerization [22] (Figure 5). In these assays, the inhibition of Arp2/3 correlated with the saturation of actin by α -catenin. Moreover, in an actin-pelleting assay, increasing amounts of α -catenin were able to compete with Arp2/3 from actin. Thus α -catenin can compete with Arp2/3 for actin filaments. In titration experiments, 5 μ M α -catenin dimer could completely suppress Arp2/3, whereas little suppression was observed with between 0.1 and 1 μ M α -catenin (Figure 5). To assess the physiological relevance of these values, we estimated the cytosolic concentration of α -catenin by preparing extracts from a known volume of MDCK cells and comparing the α -catenin signal on Western blots with known amounts of recombinant α -catenin. These experiments yielded an estimate of 0.6 μ M for the cytosolic concentration of α -catenin [22]. Thus the bulk cytosolic concentration of α -catenin is too low to inhibit Arp2/3.

These observations can be synthesized into a model that explains the available data on α -catenin and the role of Arp2/3 in cell-contact formation [22] (Figure 6). Transient contacts of cadherins on overlapping lamellipodia from two cells followed by diffusion leads to the observed clustering of cadherins in the contacts. Since β -catenin is always associated with cell-surface cadherins, cadherin clustering implies clustering of β -catenin on the cytoplasmic side of the nascent contact. This high local concentration of β -catenin recruits α -catenin from the cytosol, which can exchange readily between the membrane and proximal cytosol to provide a high local concentration of α -catenin in the cytosol under the membrane. This pool would probably dimerize and bind to actin. Note that a modest 10-fold increase in the local α -catenin concentration would suffice to completely eliminate Arp2/3 activity. In this way, branched actin polymerization, hence lamellipodial movement, would cease, consistent with the observed changes in membrane dynamics and actin organization at mature contacts. Because α -catenin can bundle actin [13,14], it might also have a positive role in promoting formation of the linear cable structures observed at contacts. Moreover, a recent report suggests that α -catenin might stimulate the activity of formins, which promote linear actin polymerization [28].

Although several aspects of this model remain to be tested, it can both explain the essential role of α -catenin in cell-contact formation and provide a mechanism for the change in membrane activity and associated actin cytoskeleton. Clearly, direct measurement of the changes in concentration is an essential experiment needed to confirm the model. What is not clear is how the cytoskeleton remains associated with the junction if the cadherin–catenin complex is not directly involved in this process. It is possible that other membrane-associated proteins are involved in the attachment to the actomyosin complex during periods of constriction associated with cell-shape changes, or that some of the candidate proteins such as vinculin can mediate the linkage to actin under certain circumstances [29,30].

Acknowledgments

This work was supported by grants GM56169 (to W.I.W.) and GM35527 (to W.J.N.) from the U.S. National Institutes of Health.

Abbreviations used

Arp2/3	actin-related protein 2/3
FLIP	fluorescence loss in photobleaching

FRAP	fluorescence recovery after photobleaching
GFP	green fluorescent protein
MDCK	Madin–Darby canine kidney

References

1. Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol.* 1963; 17:375–412. [PubMed: 13944428]
2. Hirano S, Nose A, Hatta K, Kawakami A, Takeichi M. Calcium-dependent cell–cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. *J Cell Biol.* 1987; 105:2501–2510. [PubMed: 3320048]
3. Lubarsky B, Krasnow MA. Making and shaping biological tubes. *Cell.* 2002; 112:19–28. [PubMed: 12526790]
4. Ehrlich JS, Hansen MD, Nelson WJ. Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell–cell adhesion. *Dev Cell.* 2002; 3:259–270. [PubMed: 12194856]
5. Chu YS, Thomas WA, Eder O, Pincet F, Perez E, Thiery JP, Dufour S. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. *J Cell Biol.* 2004; 167:1183–1194. [PubMed: 15596540]
6. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. *Cell.* 2003; 112:453–465. [PubMed: 12600310]
7. Adams CL, Chen YT, Smith SJ, Nelson WJ. Mechanisms of epithelial cell–cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin–green fluorescent protein. *J Cell Biol.* 1998; 142:1105–1119. [PubMed: 9722621]
8. Hirokawa N, Keller TC 3rd, Chasan R, Mooseker MS. Mechanism of brush border contractility studied by the quick-freeze, deep-etch method. *J Cell Biol.* 1983; 96:1325–1336. [PubMed: 6601660]
9. Ozawa M, Baribault H, Kemler R. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* 1989; 8:1711–1717. [PubMed: 2788574]
10. Choi HJ, Huber AH, Weis WI. Thermodynamics of β -catenin-ligand interactions: the roles of the N- and C-terminal tails in modulating binding affinity. *J Biol Chem.* 2006; 281:1027–1038. [PubMed: 16293619]
11. Aberle H, Butz S, Stappert J, Weissig H, Kemler R, Hoschuetzky H. Assembly of the cadherin–catenin complex *in vitro* with recombinant proteins. *J Cell Sci.* 1994; 107:3655–3663. [PubMed: 7706414]
12. Pokutta S, Weis WI. Structure of the dimerization and β -catenin binding region of α -catenin. *Mol Cell.* 2000; 5:533–543. [PubMed: 10882138]
13. Pokutta S, Drees F, Takai Y, Nelson WJ, Weis WI. Biochemical and structural definition of the 1-afadin- and actin-binding sites of α -catenin. *J Biol Chem.* 2002; 277:18868–18874. [PubMed: 11907041]
14. Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS. α_1 (E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. *Proc Natl Acad Sci USA.* 1995; 92:8813–8817. [PubMed: 7568023]
15. Hinck L, Nathke IS, Papkoff J, Nelson WJ. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. *J Cell Biol.* 1994; 125:1327–1340. [PubMed: 8207061]
16. Chen YT, Stewart DB, Nelson WJ. Coupling assembly of the E-cadherin/ β -catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. *J Cell Biol.* 1999; 144:687–699. [PubMed: 10037790]

17. Huber AH, Stewart DB, Laurents DV, Nelson WJ, Weis WI. The cadherin cytoplasmic domain is unstructured in the absence of β -catenin: a possible mechanism for regulating cadherin turnover. *J Biol Chem.* 2001; 276:12301–12309. [PubMed: 11121423]
18. Huber AH, Weis WI. The structure of the β -catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by β -catenin. *Cell.* 2001; 105:391–402. [PubMed: 11348595]
19. Aberle H, Schwartz H, Hoschuetzky H, Kemler R. Single amino acid substitutions in proteins of the *armadillo* gene family abolish their binding to α -catenin. *J Biol Chem.* 1996; 271:1520–1526. [PubMed: 8576147]
20. Bakolitsa C, de Pereda JM, Bagshaw CR, Critchley DR, Liddington RC. Crystal structure of the vinculin tail suggests a pathway for activation. *Cell.* 1999; 99:603–613. [PubMed: 10612396]
21. Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin–catenin–actin complex. *Cell.* 2005; 123:889–901. [PubMed: 16325582]
22. Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI. α -Catenin is a molecular switch that binds E-cadherin/ β -catenin and regulates actin filament assembly. *Cell.* 2005; 123:903–915. [PubMed: 16325583]
23. Vaezi A, Bauer C, Vasioukhin V, Fuchs E. Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev Cell.* 2002; 3:367–381. [PubMed: 12361600]
24. Kovacs EM, Goodwin M, Ali RG, Paterson AD, Yap AS. Cadherin-directed actin assembly: E-cadherin physically associates with the Arp2/3 complex to direct actin assembly in nascent adhesive contacts. *Curr Biol.* 2002; 12:379–382. [PubMed: 11882288]
25. Lien WH, Klezovitch O, Fernandez TE, Delrow J, Vasioukhin V. α -E-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. *Science.* 2006; 311:1609–1612. [PubMed: 16543460]
26. Vasioukhin V, Bauer C, Begenstein L, Wise B, Fuchs E. Hyperproliferation and defects in epithelial polarity upon conditional ablation of α -catenin in skin. *Cell.* 2001; 104:605–617. [PubMed: 11239416]
27. Abe K, Chisaka O, Van Roy F, Takeichi M. Stability of dendritic spines and synaptic contacts is controlled by α -N-catenin. *Nat Neurosci.* 2004; 7:357–363. [PubMed: 15034585]
28. Kobiela A, Pasolli HA, Fuchs E. Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. *Nat Cell Biol.* 2004; 6:21–30. [PubMed: 14647292]
29. Gates J, Peifer M. Can 1000 reviews be wrong? Actin, α -catenin, and adherens junctions. *Cell.* 2005; 123:769–772. [PubMed: 16325573]
30. Weis WI, Nelson WJ. Re-solving the cadherin–catenin–actin conundrum. *J Biol Chem.* 2006; 281:35593–35597. [PubMed: 17005550]

Figure 1. Standard model of junction organization

The cytoplasmic domain of classical cadherins binds to β -catenin. β -Catenin in turn binds to α -catenin, which binds to F-actin either directly or through other actin-binding proteins.

Figure 2. Primary structure of α -catenin, with vinculin-homology regions and binding sites for various partners indicated

Numbers are amino acid positions. ZO-1, zonula occludens 1.

Figure 3. α -Catenin homodimer structure and binding to β -catenin

(A) Crystal structure of the homodimerization domain, with protomers coloured red and turquoise. (B) Schematic illustration of β -catenin binding to α -catenin 57–264. The homodimerization domain 82–264 is shown in yellow, and the flexibly linked α -catenin 57–81 sequence in blue. The α -catenin-binding sequence of β -catenin, residues 118–149, forms a helix and is shown in red. (C) Diagram of the $\beta\alpha$ -catenin chimaera used to form a stable β -catenin– α -catenin heterodimer, coloured as in (B). (D) Crystal structure of the $\beta\alpha$ -catenin chimaera, coloured as in (B). Adapted from Mol. Cell, **5**, Pokutta, S. and Weis, W.I., ‘Structure of the dimerization and β -catenin binding region of α -catenin’, 533–543, © 2000, with permission from Elsevier.

Figure 4. Summary of FRAP parameters for E-cadherin, β -catenin, α -catenin and actin
 Ecad, E-cadherin–GFP; Ecad Δ c, E-cadherin–GFP with the cytoplasmic domain deleted;
 α cat, α -catenin–GFP; α cat Δ c, α -catenin–GFP with the actin-binding domain deleted; β cat,
 β -catenin–GFP; Actin, actin–GFP; Rh-actin, rhodamine-labelled actin microinjected into
 cells. Results are means $\pm$ S.E.M. Adapted from Cell, **123**, Yamada, S., Pokutta, S., Drees,
 F., Weis, W.I. and Nelson, W.J. ‘Deconstructing the cadherin–catenin–actin complex’, 889–
 901, © 2005, with permission from Elsevier.

Figure 5. α -Catenin can suppress Arp2/3-mediated actin polymerization

Increasing amounts of α -catenin were added to a solution containing pyrene-labelled actin and 50 nM Arp2/3 and the stimulatory WASP (Wiskott–Aldrich syndrome protein) VCA (verprolin homology, cofilin homology and acidic) domain. Polymerization results in an increase in fluorescence. Adapted from Cell, **123**, Drees, F., Pokutta, S., Yamada, S., Nelson, W.J. and Weis, W.I. ' α -Catenin is a molecular switch that binds E-cadherin/ β -catenin and regulates actin filament assembly', 903–915, © 2005, with permission from Elsevier.

Figure 6. Model for cell–cell contact formation

Transient contacts of cadherins on lamellipodia (upper left) lead to clustering of the cadherin– β -catenin complex (upper right) that recruits α -catenin. Exchange of α -catenin between cadherin-bound β -catenin and cytosol results in high local concentration of α -catenin, favouring dimerization (right). Dimeric α -catenin near the membrane can locally suppress Arp2/3 and shut off lamellipodial movement, generating a stable contact. The α -catenin may also help to cross-link actin and promote linear cable formation through formins.