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Can 1000 Reviews Be Wrong? Actin, α-Catenin, and Adherens Junctions

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Coupling between cell adhesion and the actin cytoskeleton is thought to require a stable link between the cadherin-catenin complex and actin that is mediated by α-catenin. In this issue of *Cell*, the Weis and Nelson groups call this static model into question, showing that α-catenin can directly regulate actin dynamics (Drees et al., 2005; Yamada et al., 2005).

Teaching 120 undergraduates the essentials of cellular and developmental biology in one semester is a daunting prospect. In one lecture, we cover all aspects of cell-cell and cell-matrix adhesion, so it's quite a roller-coaster ride. However, survey courses like this do reveal the essential facts of the discipline. One of these is that cadherin-based cell-cell adherens junctions mediate adhesion and are responsible for anchoring the actin cytoskeleton. If you pick up any undergraduate cell biology textbook, you can see "how things work" (Figure 1): cadherins mediate homophilic adhesion and the cytoplasmic tails of cadherin *cis*-dimers bind to intracellular proteins; β-catenin and the related protein p120 bind to the tail directly at different sites, and α-catenin then binds to β-catenin; and finally, actin filaments bind directly to α-catenin. In this issue of *Cell*, work from the Weis and Nelson groups shakes up this traditional image of the adherens junction (Drees et al., 2005; Yamada et al., 2005). They show that α-catenin, rather than being a stable link to actin, may instead act as a key regulator of actin dynamics.

How did this static view become accepted as "the way junctions work"? Adherens junctions (AJs) were first identified as electron-dense structures near the apical end of the lateral cell interface in epithelial cells. Beneath them, the actin cytoskeleton is organized into a belt of bundled actin filaments that runs around the apical end of the cell (Figure 1A). The molecular machinery that comprises AJs has been identified over the past 20 years. Cadherins were established as homophilic adhesion molecules in both cultured cells and early mouse embryos. Subsequently, the catenins were identified as proteins that coimmunoprecipitate with cadherins. The catenins are a set of both related and unrelated proteins. Of these, β-catenin is a member of the Armadillo-repeat superfamily. It binds to a conserved sequence in the distal part of the cadherin tail. p120 is distantly related to β-catenin and binds to the juxtamembrane region of cadherins. α-catenin is a distant relative of vinculin, an actin binding protein. Biochemical data suggest that E-cadherin and β- and α-catenin form a complex that is roughly stoichiometric and can be isolated from cells even under relatively harsh conditions (Ozawa and Kemler, 1992), although, under other conditions, α-catenin can be preferentially dislodged from the complex.

Genetic data support central roles for fruit-fly DE-cadherin, mouse E-cadherin, and fly β-catenin (Armadillo) in cell adhesion and the architecture of epithelial tissues. For example, mice lacking either E-cadherin or α-E-catenin (the family members expressed in epithelia) have very similar phenotypes (reviewed in Jamora and Fuchs, 2002). Both mutations disrupt the trophectodermal epithelium of the early embryo prior to implantation.

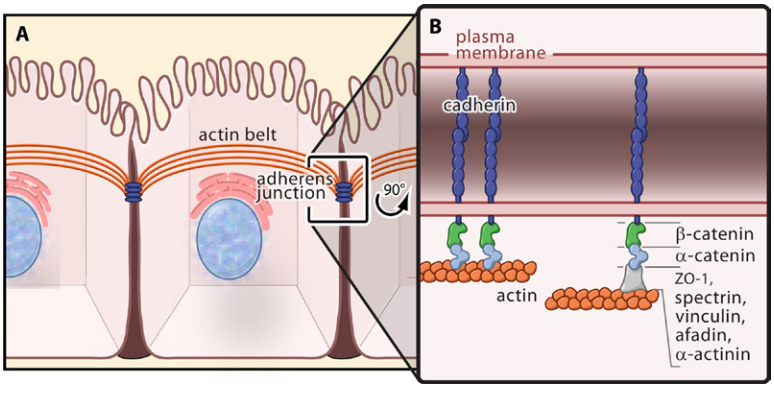

Figure 1. A Textbook Model for How the Adherens Junction Complex Connects with Actin
(A) Epithelial cells are joined by adherens junctions, positioned near the apical end of the lateral cell interface. Belts of actin filaments underlie adherens junctions.
(B) Cadherins mediate homophilic adhesion. The cytoplasmic tails of cadherin *cis*-dimers bind to intracellular proteins. β-catenin binds to the tail directly, and α-catenin then binds to β-catenin. Actin filaments then bind to α-catenin. In this model, cadherins are linked directly to actin via the catenins. The catenins could also mediate interactions to actin via binding to proteins such as ZO-1, spectrin, vinculin, afadin, and α-actinin.

AJs and the underlying actin belt are interdependent. Disruption of fruit-fly β-catenin leads to widespread defects in the polarity of the actin cytoskeleton (Cox et al., 1996), suggesting that AJs play a key role in maintaining this actin belt. Likewise, disruption of the cortical-actin cytoskeleton disrupts AJs (Quinlan and Hyatt, 1999). The current view that this interdependence relies on a direct interaction emerged from a series of biochemical experiments. The three regions of vinculin homology in α-catenin are referred to as the VH1, VH2, and VH3 domains. Biochemical data have shown that the VH1 domain binds to β-catenin, whereas the VH3 domain binds to actin (Rimm et al., 1995). α-catenin could also interact with actin via a dazzling array of binding partners, including α-actinin, vinculin, spectrin, zonula occludins-1 (ZO-1), and afadin (Figure 1B). Although the cadherin-catenin complex bound to actin has never been isolated, these biochemical and genetic data seem to provide ample support for the textbook view that the catenins provide a stable tether between cadherin and the actin cytoskeleton.

The Weis and Nelson groups (Drees et al., 2005; Yamada et al., 2005) bring complementary approaches to the subject. The Nelson lab was one of the first to establish the role of cadherins in the polarity of epithelial cells and has contributed key information about the assembly and disassembly of AJs. The Weis lab solved the structures of domains of many proteins involved in cell adhesion, including those in β- and α-catenin. Their work has led to a new view of the relationship between cadherins, catenins, and actin.

The first tenet of the current model (Figure 1B) tested by Weis and Nelson is the idea that the actin cytoskeleton is physically and stably linked to the cadherin-catenin complex (Yamada et al., 2005). As they point out, the current model is based on the "commutative property" applied to biochemistry—if E-cadherin and β-catenin bind to α-catenin and α-catenin binds to actin, then E-cadherin must bind to actin. In a series of direct tests, they show that this is not accurate. They offer compelling data suggesting that, although both binary interactions can be observed, α-catenin assembled into the cadherin-catenin complex does not bind to actin. This is demonstrated both in biochemical assays and in a new assay where cadherin-catenin complexes are assembled onto isolated patches of plasma membrane. Furthermore, neither vinculin nor α-actinin can mediate this interaction. Finally, using FRAP (fluorescence recovery after photobleaching), they show that cortical actin is much more dynamic than the cadherin-catenin complex, a finding contrary to the existence of a highly stable connection (Figure 1B).

If α-catenin does not provide a stable connection between AJs and actin, then what does? One possibility is that there is no direct or indirect connection between actin and AJs, either stable or transient. However, several observations make this unlikely. Although the circumferential belt of actin underlying AJs could be maintained by proteins whose concentration is locally raised by transient association with the AJ complex, this would not explain why altering the actin cytoskeleton destabilizes AJs. Likewise, if the AJ is not tethered to actin, how does contraction of the actin belt during apical constriction trigger cell constriction during embryonic development (for example, during neural-tube formation and mesoderm invagination)? In the fruit fly *Drosophila*, Wieschaus and colleagues recently showed that AJs are repositioned in response to actin-based apical constriction during mesoderm invagination, and they demonstrate that reducing AJ function by depleting β-catenin allows the actin-myosin ring to contract without changing a cell's shape, strongly supporting the existence of some kind of physical link between actin and AJs (Dawes-Hoang et al., 2005).

A second possibility is that other molecules mediate a direct connection. One attractive candidate is the nectin-afadin system (Figure 2B; reviewed in Takai and Nakanishi, 2003). Nectins are immunoglobulin-superfamily adhesion molecules that also are localized to AJs and bind to afadin. Afadin is a cytoplasmic plaque protein that binds to actin as well as to several actin-associated proteins and thus could serve as an alternate link. It is also possible that actin binding proteins other than those tested by the Weis and Nelson groups may be recruited to the cadherin-catenin complex. However, the FRAP data would suggest that none of these interactions is stable.

A third possibility is that the link is mediated by many weak and transient interactions (Figure 2C), which cannot be detected biochemically. Interestingly, a similar model has been proposed for the function of the cadherin extracellular domain. Cadherins interact homophilically with the cadherins of neighboring cells, mediating cell-cell adhesion. The current model suggests that the individual cadherin-cadherin bond is relatively weak. However, given that many cadherins are clustered into AJs, the sum of these weak, transient interactions creates an adhesive interface that can be strong yet easily remodeled.

Without a stable linkage between AJs and actin, one is still left with the fact that all three core components of AJs are essential for adhesion and for the polarization of the actin cytoskeleton in epithelial cells. Moreover, site-specific mutations in the α-catenin binding site of fly β-catenin disrupt adhesion and polarity (Orsulic and Peifer, 1996), suggesting that recruitment of α-catenin into the cadherin-catenin complex is also essential. If α-catenin does not provide a stable link to AJs, what role does it fulfill? Nelson, Weis, and their colleagues suggest that α-catenin acts as a molecular switch that regulates actin dynamics at AJs.

How might α-catenin act as a molecular switch? α-catenin can exist as a monomer, a homodimer, and in heterodimers with β-catenin. Both homodimerization and interaction with β-catenin occur via its N-terminal VH1 domain. Thus, homodimerization and heterodimerization compete with one another. In contrast, the interaction with actin involves the C-terminal VH3 domain. In a simple world, this would mean that actin binding and AJ association would be independent. However, the Nelson group (Yamada et al., 2005) now demonstrates that α-catenin cannot simultaneously bind to β-catenin and actin. How can this be? In their study, the Weis lab (Drees et al., 2005) provides evidence that α-catenin behaves in an allosteric fashion, with its affinity for

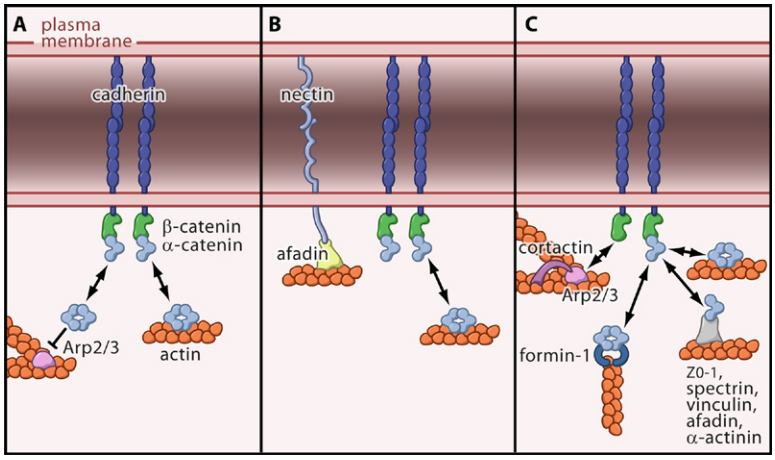

Figure 2. Revised Models for How the Adherens Junction Complex Connects with Actin

(A) The model proposed by Weis and Nelson: an α-catenin monomer binds to β-catenin; α-catenin homodimers, released from cadherin-catenin complexes, bind to actin and antagonize Arp2/3 function.

(B and C) Alternative models explain the association between actin and adherens junctions.

(B) A direct connection to actin is mediated by other junction proteins, such as nectin and afadin.

(C) The connection is mediated by the cumulative effect of several weak transient interactions between actin binding proteins (such as ZO-1, spectrin, vinculin, afadin, and α-actinin) and adherens junction components, facilitated by cadherin clustering.

one ligand affected by binding to another. The α-catenin-β-catenin heterodimer has a high affinity for cadherin but has reduced affinity for actin, whereas dimeric α-catenin cannot bind to the cadherin-catenin complex but has a high affinity for actin (Figure 2A). This suggests that interactions at the N terminus somehow modulate the actin affinity of the C terminus of α-catenin, thereby allowing α-catenin to switch between different states.

Drees et al. (2005) next consider what roles α-catenin might have when it is not in the cadherin-catenin complex. Because α-catenin homodimers bind to actin, the authors speculate that α-catenin may influence actin dynamics at AJs. To test this possibility, they examined whether α-catenin can influence actin polymerization mediated by the Arp2/3 complex (containing actin-related proteins 2 and 3). The Arp2/3 complex nucleates actin polymerization from the sides of existing filaments, which creates a branched, dendritic actin array. Using a pyrene-actin fluorescence assay, they found that the actin polymerization that is normally observed in the presence of the Arp2/3 complex and the activation domain of the Wiskott-Aldrich syndrome protein (WASP) was suppressed by the addition of the α-catenin homodimer. The degree of suppression is concentration dependent and correlates with the binding of the α-catenin homodimer to actin. Interestingly, the amount of Arp3 bound to actin decreases when the concentration of the α-catenin homodimer is increased, suggesting that the α-catenin homodimer competes with the Arp2/3 complex for binding to actin filaments. α-catenin might inhibit actin branching, facilitating formation of the belt of unbranched actin filaments (Figure 2A).

One caveat to this analysis is that the suppression of the Arp2/3 complex requires very high concentrations of the α-catenin homodimer, with mild effects observed at 1 μM and complete suppression requiring 7.5 μM. In contrast, concentrations only in the nM range of the vasodilator-stimulated phosphoprotein (VASP) are needed to increase the rate of actin polymerization from spectrin/F-actin seeds in the presence of capping proteins (Barzik et al., 2005). The requirement for such a high concentration of the α-catenin homodimer raises questions about the physiological relevance of this result. However, Drees et al. (2005) found that α-catenin bound to E-cadherin-β-catenin complexes can leave this complex and bind to actin in solution. Therefore, they propose that E-cadherin and β-catenin recruit α-catenin to AJs. However, because this interaction is transient, the subsequent dissociation of α-catenin from this complex leads to a local increase in the concentration of α-catenin in the vicinity of apical actin. Whether this is sufficient to create the concentration needed to allow α-catenin to influence Arp2/3-mediated actin polymerization at AJs remains to be determined. A recent study examining the local concentration of several actin regulators in fission yeast shows that techniques to address this are now available (Wu and Pollard, 2005).

However, α-catenin is not the only regulator of actin dynamics found at the AJ. To date, several actin regulators have been shown to localize to either established or nascent AJs (Figure 2C), including Ena/VASP proteins (Vasioukhin et al., 2001), formin-1 (Kobielak et al., 2004), the Arp2/3 complex (Kovacs et al., 2002), and its activator cortactin (Helwani et al., 2004). The Arp2/3 complex and formin-1 can each nucleate actin filaments, although the geometry of the resulting filaments differs. Formin-1 nucleates polymerization of linear actin filaments, whereas the Arp2/3 complex nucleates polymerization of branched actin filaments. Ena/VASP proteins promote the continued elongation of existing filaments by binding to the quickly growing barbed end and preventing the binding of capping proteins (Barzik et al., 2005). The localization of both Ena/VASP proteins and formin-1 to AJs is dependent on α-catenin (Kobielak et al., 2004; Vasioukhin et al., 2000). Furthermore, formin-1 can directly interact with α-catenin (Kobielak et al., 2004), although it is not known whether α-catenin can bind to both β-catenin and formin-1 at the same time. Interestingly, both cortactin (Helwani et al., 2004) and a component of the Arp2/3 complex, p34 (Kovacs et al., 2002), coimmunoprecipitate with E-cadherin, suggesting that their AJ localization may depend on a direct or indirect association with the cadherin-catenin complex. These actin regulatory proteins

may provide a molecular toolkit that can dynamically alter the state of actin at AJs. Dynamic changes in actin occur as AJs are assembled, suggesting that this process is highly regulated. In order to enable diverse responses, perhaps cells can regulate the release of distinct subsets of the actin regulators that are localized to AJs depending on the situation. For example, formins may be involved in the formation of linear actin cables at nascent junctions. These additional players might also help account for the very rapid polymerization of actin induced by cadherin clustering, as observed by several labs. Likewise, regulators of the microtubule cytoskeleton might localize at AJs, helping to explain the effects of cell adhesion on microtubules.

The Weis and Nelson model suggests that α-catenin continuously shuttles between an "inactive" cadherin bound pool and an actin bound pool that is active in regulation of the cytoskeleton (Figure 2A). One challenge to this model comes from the analysis of fusion proteins that covalently link E-cadherin and α-catenin, eliminating the need for β-catenin as a bridge. Fusion proteins that join E-cadherin (minus its β-catenin binding site) to either full-length α-catenin or the α-catenin VH3 domain each rescue cell adhesion (Nagafuchi et al., 1994). Furthermore, they also confer resistance to extraction by nonionic detergents, a common assay for cytoskeletal association, and disrupting the actin cytoskeleton interferes with the strong adhesion mediated by these fusion proteins. These data suggest that if α-catenin acts as an allosteric regulator of the cytoskeleton, it may be able to do so when recruited to AJs by covalent linkage to E-cadherin. This could relieve the allosteric repression conferred by β-catenin binding. However, this work was done in the presence of wild-type α-catenin. Wild-type α-catenin might also be recruited to AJs by homodimerization with the α-catenin-E-cadherin fusion protein and may be able to regulate actin. Although E-cadherin-α-catenin fusion proteins rescued adhesion, dividing cells did not round up, and cell motility within an epithelial sheet was impaired (Nagafuchi et al., 1994), suggesting that AJs built around this fusion protein may not allow for rapid remodeling.

Recent work extends these findings in vivo. Pacquelet and Rorth (2005) examined the ability of DE-cadherin-α-catenin fusion proteins to carry out an array of functions during *Drosophila* oogenesis. Fusions similar to those studied by Nagafuchi et al. (1994) fully rescue the adhesive functions for DE-cadherin in both the germline and somatic follicle cells. However, these fusion proteins do not rescue DE-cadherin-dependent migration of one group of somatic follicle cells, the border cells (Niewiadomska et al., 1999). But this function is rescued if the juxtamembrane region of DE-cadherin is included in the fusion protein. This region binds to p120, a distant relative of β-catenin. Thus, in vivo, DE-cadherin-α-catenin fusion proteins can restore virtually all of the known functions of DE-cadherin. This may limit the generality of the allosteric regulation model but is also subject to the caveat that these experiments were conducted in the presence of wild-type α-catenin. It will be of interest to test the function of these fusion proteins in flies carrying mutations in α-catenin, when such mutants become available.

In addition to regulating the actin cytoskeleton, α-catenin may have other unexpected roles in the biology of epithelial cells. One striking example is provided by work from the Fuchs group, in which α-E-catenin was knocked out in the skin cells of mice (Vasioukhin et al., 2001). This led to expected defects in cellular junctions and severe defects in the organization of the skin. However, there were some surprising consequences: skin cells underwent hyperproliferation both in vivo and in vitro, and the MAP kinase pathway was constitutively activated. Perhaps, like its binding partner β-catenin, α-catenin has a dual signaling function, with perhaps some role in the nucleus.

It is encouraging to those of us still engaged in studying adhesion that 20 years of research on its molecular mechanisms raises many exciting new questions, just as old questions have been answered. We look forward to seeing what the textbooks will say about AJs in 20 years' time.

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