α-Catenin Is a Molecular Switch that Binds E-Cadherin-β-Catenin and Regulates Actin-Filament Assembly

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SUMMARY

Epithelial cell-cell junctions, organized by adhesion proteins and the underlying actin cytoskeleton, are considered to be stable structures maintaining the structural integrity of tissues. Contrary to the idea that α-catenin links the adhesion protein E-cadherin through β-catenin to the actin cytoskeleton, in the accompanying paper we report that α-catenin does not bind simultaneously to both E-cadherin-β-catenin and actin filaments. Here we demonstrate that α-catenin exists as a monomer or a homodimer with different binding properties. Monomeric α-catenin binds more strongly to E-cadherin-β-catenin, whereas the dimer preferentially binds actin filaments. Different molecular conformations are associated with these different binding states, indicating that α-catenin is an allosteric protein. Significantly, α-catenin directly regulates actin-filament organization by suppressing Arp2/3-mediated actin polymerization, likely by competing with the Arp2/3 complex for binding to actin filaments. These results indicate a new role for α-catenin in local regulation of actin assembly and organization at sites of cadherin-mediated cell-cell adhesion.

INTRODUCTION

Epithelial cell-cell junctions are organized by adhesion proteins and the underlying actin cytoskeleton. They provide adaptable interfaces that can respond to signals for cell movement during convergent extension in gastrulation (Keller, 2002) or changes in cell shape during tube formation (Lubarsky and Krasnow, 2002) but also provide a constant permeability barrier between different biological compartments in the body. Analysis of how migrating cells initiate cell-cell adhesion has revealed dramatic changes in membrane dynamics and organization of the actin cytoskeleton. Migrating cells have characteristic forward-moving lamellipodia produced by rapid Arp2/3-nucleated assembly of a branched actin network perpendicular to the leading edge (Pollard and Borisy, 2003). Upon cell-cell adhesion, lamellipodial activity is reduced over the contacting area, and there is a concomitant reorganization of actin filaments (Adams et al., 1998; Ehrlich et al., 2002; Omelchenko et al., 2001; Vaezi et al., 2002; Vasioukhin et al., 2001); electron microscopy of simple epithelial cells indicates the formation of bundles of actin filaments parallel to the contacting membranes (Hirokawa et al., 1983), an organization very different from that of branched actin in lamellipodia. It is unknown how engagement of cadherins between migrating cells causes these dramatic changes in actin-filament assembly and organization. The intracellular domain of cadherins binds cytoplasmic proteins that are thought to recruit and organize actin filaments (Gumbiner, 2000; Jamora and Fuchs, 2002). These molecular linkages (Nagafuchi and Takeichi, 1988; Ozawa et al., 1989) include high-affinity binding of β-catenin to the cadherin cytoplasmic domain (Huber et al., 2001) and a lower-affinity interaction between β-catenin and α-catenin (Pokutta and Weis, 2000). Since α-catenin can also bind actin filaments in vitro (Pokutta et al., 2002; Rimm et al., 1995), it is widely accepted that α-catenin binds to the cadherin-β-catenin complex bridges these components to actin. In addition, many actin binding proteins have been reported to bind α-catenin, including vinculin and α-actinin (Kobiela and Fuchs, 2004), suggesting that they could also link the cadherin-catenin complex to the actin cytoskeleton. Furthermore, the Rho family of small GTPases (Braga, 2002; Fukata and Kaibuchi, 2001) and the actin nucleators formin (Kobiela et al., 2004) and the Arp2/3 complex (Kovacs et al., 2002) are involved in

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cell-cell adhesion and may regulate actin dynamics around the cadherin-catenin complex.

In the accompanying paper (Yamada et al., 2005 [this issue of Cell]), experiments with purified proteins demonstrated that the ternary complex of cadherin-β-catenin-α-catenin does not bind directly to actin filaments or indirectly through vinculin or α-actinin. Moreover, live-cell imaging showed that the cadherin-catenin complex has dynamics that are very different from those of actin, consistent with the lack of a stable linkage between the cadherin-catenin complex and actin in cells.

The inability of α-catenin to bind simultaneously to the cadherin-β-catenin complex and actin indicates that it may function as a molecular switch, whereby binding to one partner changes the ability to interact with the other. Here we provide evidence that these changes are associated with distinct conformational states of α-catenin and that dimerization of α-catenin influences its ability to selectively bind to β-catenin or actin. Given the highly dynamic properties of the actin network at cell-cell contacts, we further examined the role of α-catenin in regulating actin assembly. We show that α-catenin suppresses actin polymerization by the Arp2/3 complex, suggesting that assembly and clustering of the cadherin-catenin complex at cell-cell contacts may provide a pool of α-catenin that can locally regulate actin dynamics and organization.

RESULTS

Native and Recombinant α-Catenin Exist as Monomer and Homodimer

Purified α-catenin has been reported to be a homodimer in solution (Koslov et al., 1997). The crystal structure of the N-terminal domain of α-catenin revealed that the dimerization domain overlaps the β-catenin binding domain (Pokutta and Weis, 2000). Since the stoichiometry of the α-catenin-β-catenin heterodimer is 1:1, the α-catenin homodimer would have to dissociate before α-catenin could bind β-catenin (Pokutta and Weis, 2000). Thus, the molecular state of α-catenin (monomer, homodimer, heterodimer with β-catenin) appears to be critical for interactions with potential binding partners.

A pool of α-catenin is found in MDCK cell cytoplasm, and another is associated with the E-cadherin-β-catenin complex at the plasma membrane (Hinck et al., 1994). Fractionation of a 100,000 × g cytosol supernatant by gel filtration revealed two peaks of α-catenin (Figure 1A) with elution volumes similar to those of purified recombinant His-tagged α-catenin (Figure 1B). The molecular mass of the two peaks of His-tagged α-catenin, determined by multiangle light scattering (MALD), corresponded to an α-catenin monomer and homodimer, respectively (Figure 1B). Data from several independent experiments indicated that 60%–75% of cytosolic α-catenin in MDCK cells was monomeric (Figure 1A), and the proportion of monomer was independent of the cell plating density (data not shown).

Although the first peak of α-catenin observed in the cytosol fractionation can be superimposed on the elution profile of the recombinant α-catenin homodimer, we found that endogenous β-catenin also coeluted with this peak. Since recombinant β-catenin elutes at a smaller apparent molecular weight (e.g., Figures 2A and 2B), the observed coelution of β-catenin and α-catenin may indicate that there is a β/α-catenin heterodimer in MDCK cell cytosol. Indeed, α-catenin coimmunoprecipitated with β-catenin from the higher-molecular-weight peak (Figure 1C). However, a β/α-catenin heterodimer does not account for all α-catenin in this peak, as endogenous α-catenin can be coimmunoprecipitated with a green fluorescent protein-α-catenin fusion protein (GFP-α-catenin), indicating the presence of α-catenin homodimer in cytosol (Figure 1C). Comparison of the amount of α-catenin in the pellet and supernatant after coimmunoprecipitation with β-catenin indicates that approximately 20% of α-catenin in the higher-molecular-weight fraction was bound to β-catenin, with approximately 80% an α-catenin homodimer. Thus, in contrast to previous reports, purified α-catenin can exist as a monomer and homodimer in solution, and most of endogenous α-catenin in MDCK cell cytosol is monomeric.

The concentration of α-catenin in the MDCK cell cytosol was estimated from α-catenin Western blots by comparing the intensities of bands from cytosol to those from known amounts of input recombinant α-catenin. The most intense monomer fractions had a concentration of 7 nM. Accounting for cell volume and dilution during cell lysis and column chromatography, we obtain an estimate of 0.6 μM α-catenin in cytosol. Recombinant α-catenin appears as a mixture of monomer and dimer when run on gel filtration columns at concentrations of 2–20 μM (e.g., Figure 1B). This places the homodimerization constant in the micromolar range, which is consistent with the observation that the majority of cytosolic α-catenin is monomeric.

α-Catenin Monomer and Homodimer Show Preferential Binding to β-Catenin and Actin, Respectively

Since α-catenin forms a 1:1 complex with β-catenin and the homodimerization and β-catenin binding domains of α-catenin overlap (Pokutta and Weis, 2000), we hypothesized that α-catenin monomer should bind more readily to β-catenin. Formation of the β-catenin-α-catenin complex was monitored in solution by gel filtration chromatography. Mixtures of α-catenin and β-catenin were applied to an analytical gel filtration column at 26 μM, which then became diluted approximately 10-fold on the column. Under these conditions, α-catenin homodimer did not bind β-catenin, and both proteins eluted separately (Figure 2A), whereas the majority of α-catenin monomer eluted in a 1:1 complex with β-catenin (Figure 2B). We assessed whether the presence of E-cadherin affects the relative affinities of α-catenin monomer or homodimer for β-catenin. Pull-down assays were established using glutathione-agarose beads to capture bacterially expressed GST-E-cadherin cytoplasmic domain (GST-Ecys), full-length recombinant β-catenin, and increasing amounts of full-length His-tagged α-catenin monomer or homodimer; note that recombinant GST-Ecys and β-catenin bind with 36 nM affinity in a 1:1 stoichiometry in solution.
The resulting bead bound complexes showed that the α-catenin monomer has a higher apparent affinity for β-catenin than the homodimer (saturation reached at approximately 20 μM monomer versus 50 μM for the homodimer) (Figure 2C). Gel filtration and pull-down assays using plakoglobin, a homolog of β-catenin that also binds E-cadherin and α-catenin, gave similar results (see Figure S1 in the Supplemental Data available with this article online).

We tested whether α-catenin monomer and homodimer have different apparent affinities for actin filaments in an in vitro actin pelleting assay. Greater than 90% of the α-catenin homodimer pelleted with actin filaments, whereas less than 40% of the monomer bound (Figure 2D). As a fraction of purified α-catenin monomer rapidly converts to the homodimer (see above), it is impossible to obtain samples that are 100% pure monomeric α-catenin. Therefore, it is possible that the α-catenin bound to actin filaments in this experiment is due to contaminating homodimer and that the monomer does not bind actin filaments under these conditions.

In the accompanying paper (Yamada et al., 2005), we showed that binding of α-catenin to β-catenin and actin is mutually exclusive. This was confirmed by using the chimeric β-catenin–α-catenin protein, which mimics the interaction of the two proteins by covalently linking the α-catenin binding site of β-catenin to the β-catenin binding domain of α-catenin (Pokutta and Weis, 2000). Gel filtration chromatography (data not shown) and MALS (Figure 1D) demonstrated that the β-catenin–α-catenin chimera is monomeric. Therefore, the inability of the chimera to bind to actin could be
Figure 2. β-Catenin and Actin Binding Activity of α-Catenin Monomer and Dimer

(A) Superdex 200 gel filtration chromatography of the α-catenin dimer and β-catenin incubated overnight (red line) and of the individual proteins, α-catenin dimer (blue) and β-catenin (black). Fractions analyzed by SDS-PAGE are shown for the individual runs on the right. Peak fractions are indicated by colored bars.

(B) Gel filtration chromatography as described in (A) with the α-catenin monomer.

(C) GST-E-cadherin cytoplasmic domain (10 μM) and β-catenin (10 μM) were incubated with α-catenin monomer or dimer at the indicated concentrations. Protein complexes were isolated on GST-agarose beads and analyzed by SDS-PAGE. Background binding of contaminating uncleaved GST-β-catenin is seen in the β-catenin-containing samples.

(D) Sedimentation of monomeric and dimeric α-catenin in the presence and absence of F-actin. Supernatant S containing the unbound protein and pellet P containing actin-bound protein were analyzed by SDS-PAGE.
due to its oligomeric state. Alternatively, binding of β-catenin induces a conformation of α-catenin that is unable to bind actin.

The inability of α-catenin to bind both β-catenin and actin simultaneously suggests that the N- and C-terminal domains of α-catenin are allosterically coupled, whereby binding to one partner alters its ability to bind to the other. Interestingly, the homologous protein vinculin shows the opposite behavior: the head and tail regions of vinculin bind to talin and actin, respectively, only if an autoinhibitory head-tail interaction is relieved (Bass et al., 2002; Johnson and Craig, 1995). Purified vinculin behaves as a globular protein on a sizing column, whereas monomeric α-catenin displays a considerably larger apparent molecular mass (Figure 3A), indicating a more extended, open conformation. Moreover, we could not detect an interaction between the N- and C-terminal domains of α-catenin (S.P., unpublished data). The βα-catenin chimera has a gel filtration elution profile similar to that of monomeric α-catenin, implying that its inability to bind to actin is not due to an interaction between α-catenin N- and C-terminal domains. Furthermore, proteolytic-sensitivity experiments indicate that the α-catenin monomer has a conformation different from that of the βα-catenin chimera (Figure 3B). Thus, the inability of α-catenin to bind actin in the presence of β-catenin is probably due to conformational changes produced when α-catenin monomer binds to the β-catenin-E-cadherin complex. Further structural studies will be needed to analyze these conformational changes in α-catenin.

Figure 3. Conformational Properties of α-Catenin and Comparison to Vinculin
(A) Vinculin elutes at a lower apparent molecular weight on a gel filtration column than α-catenin. MDCK cell cytosol was analyzed by Superdex 200 gel filtration chromatography. Fractions were analyzed by SDS-PAGE and subsequent Western blotting for α-catenin and vinculin. Intensities of the α-catenin and vinculin bands were plotted versus the fraction number.
(B) Protolytic sensitivity of α-catenin monomer, homodimer, and β-catenin-α-catenin chimera. SDS-PAGE of α-catenin monomer, dimer, and βα-catenin chimera incubated for 0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, and 4 hr with trypsin. The asterisk indicates a degradation product unique to the α-catenin monomer. Molecular-weight markers are indicated on the left. The indicated dimerization and M domains were identified previously as comprising residues 82–264 and 385–651, respectively (Pokutta et al., 2002; Pokutta and Weis, 2000).
Exchange between Cytoplasmic and Membrane Bound Pools of $\alpha$-Catennin

The mutually exclusive binding of $\alpha$-catennin to $\beta$-catennin or actin implies that $\alpha$-catennin must dissociate from the E-cadherin-$\beta$-catennin complex before it can bind to actin. This was tested by incubating actin filaments with preassembled, gel filtration-purified cadherin-catenin complex containing a 1:1:1 molar ratio of E-cadherin cytoplasmic domain, $\beta$-catennin, and $\alpha$-catennin at a series of concentrations (Figure 4A) or for different times (Figure 4B). The actin filaments were then pelleted, and bound proteins were identified. E-cadherin or $\beta$-catennin did not pellet above background levels (see also Yamada et al., 2005). However, a significant fraction of $\alpha$-catennin dissociated from the complex and pelletted with actin filaments independently of incubation time (Figure 4B).

The data obtained from the purified, recombinant proteins show that $\alpha$-catennin can exchange between an E-cadherin-$\beta$-catennin complex and actin filaments in solution. We next tested whether this exchange occurs in cells. It was shown in the accompanying paper that GFP-labeled catennins display significant fluorescence recovery after photobleaching at the membrane (Yamada et al., 2005). Fluorescence recovery of peripheral membrane proteins can occur in two ways: lateral diffusion along the membrane surface in association with an integral membrane protein or exchange with the cytoplasmic pool. There is a significant cytoplasmic pool of $\alpha$-catennin that could exchange with membrane bound $\alpha$-catennin (Figure 4C). To test the extent of $\alpha$-catennin exchange between these pools, we measured the fluorescence loss of GFP-$\alpha$-catennin at cell-cell contacts while continuously photobleaching the cytoplasmic pool of GFP-$\alpha$-catennin (fluorescence loss in photobleaching, FLIP; Figure 4D and Movie S1). The membrane bound pool of $\alpha$-catennin, measured by subtracting the cytoplasmic fluorescence intensity from that at cell-cell contacts, decreased by an average of $\sim 10\%$ over a period of 9 min ($n = 15$; Figures 4D and 4E), suggesting that a fraction of membrane bound $\alpha$-catennin dissociated from $\beta$-catennin and entered the cytoplasm.

Since the fluorescence intensity of cytoplasmic GFP-$\beta$-catennin was near background levels (see Yamada et al., 2005), as expected (Näthke et al., 1994), we could not reliably measure the dynamics of membrane bound GFP-$\beta$-catennin. It is possible that the observed exchange of $\alpha$-catennin, as well as the recovery of GFP-tagged cadherins and catennins reported in the accompanying paper, is due to endocytosis of cadherin-catenin complexes from the membrane and fusion of cadherin-containing cytoplasmic vesicles at the
membrane. However, no vesicle fusion events that coincided with the fluorescence recovery of E-cadherin or catenins were detected, although the temporal and spatial resolution of our microscope may not be sufficient to reliably detect these events. Moreover, the half-life of E-cadherin in MDCK cells is ~5 hr (Shore and Nelson 1991), which converts to a turnover of ~1% of E-cadherin over the period that the recovery was measured. In addition, the putative recycling pool of cadherin vesicles is <2% of total E-cadherin (Le et al., 1999). Thus, these mechanisms may contribute to a small portion of z-catenin turnover, but direct exchange with the cytosolic pool is most likely to be the dominant mechanism.

z-Catenin Suppresses Arp2/3-Mediated Actin Polymerization

The mobility of the cadherin-catenin complex is quantitatively different from those of the membrane-associated and cytoplasmic pools of actin and several other actin binding proteins, observations consistent with in vitro binding experiments showing that the cadherin-catenin complex does not bind actin (Yamada et al., 2005). Nevertheless, actin is associated with cell-cell contacts, and actin dynamics are important for induction of cell-cell adhesion (Adams et al., 1998; Ehrlich et al., 2002; Vaezi et al., 2002), indicating that cadherin-mediated cell-cell adhesion somehow induces changes in actin organization and dynamics. Therefore, we examined whether z-catenin can influence Arp2/3 complex-mediated actin assembly, which plays a central role in actin assembly and branching in lamellipodia (Pollard and Borisy, 2003) and is localized to sites of initiating cell-cell contact (Kovacs et al., 2002).

Actin polymerization in the presence of z-catenin, purified Arp2/3 complex, and the activation domain (VCA) of the Wiskott-Aldrich syndrome protein (WASP- VCA) was measured in a standard pyrene-actin fluorescence assay. As expected, Arp2/3 complex alone had little effect on actin polymerization, but it strongly enhanced actin polymerization in the presence of WASP-VCA (Figure 5A; Prehoda et al., 2000). The presence of z-catenin monomer or homodimer had little or no effect on actin polymerization (data not shown). However, in the presence of the Arp2/3 complex and WASP-VCA, z-catenin monomer increased the initial lag phase of actin polymerization (Figure 5B). This effect was significantly reduced by the addition of an equimolar amount of b-catenin (Figure 5C); b-catenin alone did not have an effect on actin polymerization in the presence of Arp2/3-WASp (Figure 5D). The b2-catenin chimera (b2-cat), which mimics the b-catenin-z-catenin interaction and does not bind actin filaments (Yamada et al., 2005), also had no effect on actin polymerization induced by the Arp2/3 complex and WASP-VCA (Figure 5E).

In contrast to monomeric z-catenin, z-catenin homodimer completely suppressed actin polymerization in the presence of the Arp2/3 complex and WASP-VCA at 5 mM concentration (Figure 5F). The actin binding domain of z-catenin (z-cat 671–906), which is also a dimer (S.P., unpublished data), suppressed actin polymerization induced by Arp2/3 complex and WASP-VCA to an extent similar to that of the z-catenin homodimer (Figure 5G). It is possible that bundling of actin filaments by z-catenin homodimers (Rimm et al., 1995) sterically inhibited the Arp2/3 complex from binding to actin filaments, thereby suppressing actin polymerization. However, addition of z-actinin, another actin-bundling protein, did not suppress actin polymerization induced by the Arp2/3 complex and WASP-VCA at 5 mM concentration (Figure 5H).

z-Catenin Competes with Arp2/3 for Binding to Actin Filaments at High Concentrations

We sought to elucidate the molecular mechanism by which z-catenin suppresses Arp2/3/WASp-VCA-stimulated actin polymerization. We first tested whether z-catenin capped the growing barbed end of actin filaments or sequestered actin monomers by measuring the critical concentration for actin polymerization, which increases when the barbed end of actin filaments are capped. The critical concentration of actin in the presence and absence of 5 mM z-catenin was not significantly different (0.1 mM and 0.07 mM, respectively; Figure 6A). In the presence of z-catenin, the slope of the F-actin curve decreased, which is probably due to changes in pyrene fluorescence when z-catenin binds to actin filaments. The G-actin fluorescence curve was not significantly altered by the presence of z-catenin (Figure 6A), and G-actin does not bind to GST-z-catenin in pull-down experiments (data not shown). Finally, we asked whether suppression of actin polymerization in the presence of the Arp2/3 complex and WASP-VCA might be due to direct binding of z-catenin to, and sequestration of, the Arp2/3 complex. However, purified Arp2/3 complex did not bind to either z-catenin monomer or homodimer (Figure 6B). Thus, z-catenin does not appear to cap the growing barbed end of actin filaments or sequester either G-actin or the Arp2/3 complex.

The suppression of Arp2/3-mediated actin polymerization by z-catenin was concentration dependent (Figure 6C) and correlated with z-catenin binding to actin filaments (Figures 6D and 6E). In the pyrene-actin polymerization assay, suppression began at 1 mM and 2.5 mM and reached full inhibition at 7.5 mM and 15 mM for z-catenin homodimer and monomer, respectively. At intermediate concentrations (1–2.5 mM for homodimer; 2.5–5 mM for monomer), z-catenin increased the lag phase of actin polymerization, but actin polymerization eventually proceeded rapidly. Although the concentration of z-catenin monomer and homodimer required for complete suppression appears to differ only by a factor of two, we have to take into account that we are unable to isolate z-catenin monomer without some contaminating homodimer. Therefore, the actual difference between the suppressive activities of z-catenin monomer and homodimer is likely to be more pronounced than that observed in these experiments. Addition of b-catenin reduced the inhibitory effect of z-catenin (Figure 5C); since the z-catenin-b-catenin complex does not bind to actin filaments (Yamada et al., 2005), the presence of b-catenin reduces the effective concentration of z-catenin that can bind actin. In an actin cosegmentation assay, the amount of Arp2/3 complex bound to actin filaments decreased as the concentration of z-catenin increased (Figures 6D and 6E). Taken together, these results...
indicate that the suppressive effect of $\alpha$-catenin on Arp2/3-mediated actin polymerization is likely due to direct competition with the Arp2/3 complex for binding to actin filaments.

**DISCUSSION**

Cell-cell contacts are considered to be stable structures that maintain the structural integrity of tissues and are thought to be formed by clustering of cell-adhesion proteins through binding between opposed extracellular domains and linkage through the cytoplasmic domain to the underlying actin cytoskeleton (Gumbiner, 2000; Jamora and Fuchs, 2002). The cadherin cytoplasmic domain binds with high affinity to $\beta$-catenin (Huber et al., 2001), which in turn binds with weaker affinity to $\alpha$-catenin (Pokutta and Weis, 2000). Given that $\alpha$-catenin binds to actin filaments (Pokutta et al., 2002; Figure 5. $\alpha$-Catenin Suppresses Arp2/3-Mediated Actin Dynamics

Effect of $\alpha$-catenin, $\beta$-catenin, and $\alpha$-actinin on Arp2/3-mediated actin polymerization measured by pyrene-actin assay. Assembly reactions contained 5 $\mu$M actin containing 10% pyrene actin, 50 nM Arp2/3 complex, 50 nM WASp-VCA, and 5 $\mu$M of the indicated protein.

(A) Actin alone or in the presence of Arp2/3 complex with and without VCA.

(B–H) Arp2/3-mediated actin polymerization in the presence or absence of $\alpha$-catenin monomer (B), $\alpha$-catenin monomer and $\beta$-catenin (C), $\beta$-catenin (D), $\alpha\beta$-catenin chimera (E), $\alpha$-catenin dimer (F), $\alpha$-catenin tail domain aa 671–906 (G), or $\alpha$-actinin (H).
Rimm et al., 1995) and to other actin binding proteins such as vinculin and α-actinin (Hazan et al., 1997; Knudsen et al., 1995; Watabe-Uchida et al., 1998; Weiss et al., 1998), it was reasonable to assume that α-catenin bound to the cadherin-β-catenin complex also binds directly or indirectly to actin filaments. However, direct tests of this model failed. In the accompanying paper, we showed that α-catenin does not bind simultaneously to the cadherin-catenin complex and actin filaments (Yamada et al., 2005). These results predict that interactions between the cadherin-catenin complex and actin filaments.
complex and underlying actin cytoskeleton in cells might be very dynamic rather than being static as has been assumed.

Although direct interactions between the cadherin complex and actin filaments were not verified experimentally, there is a considerable body of work concluding that some sort of interaction of actin filaments and the cadherin-catenin complex is important in cell-cell adhesion. Cytochalasin D and latrunculin A, which change the dynamic organization of the actin cytoskeleton, reduce adhesion and weaken cell-cell contacts (Angres et al., 1996; Chu et al., 2004; Immamura et al., 1999; Matsuzaki et al., 1990). However, these drugs have global effects on actin organization that are not restricted to effects on only cell-cell contacts. Genetic deletion of z-catenin potentially provides a more direct approach to disrupt the putative cadherin-actin linkage. Cell-cell adhesion in z-catenin null cells is reduced and could be rescued by re-expression of z-catenin (Bullions et al., 1997; Hirano et al., 1992; van Hengel et al., 1997; Watabe et al., 1994; Watabe-Uchida et al., 1998). However, it is noted that cell-cell adhesion occurred in some z-catenin null cells (Maeno et al., 1999), presumably because there was sufficient cadherin on the cell surface to initiate cell-cell adhesion. z-catenin null cells have also been used to express chimeras between z-catenin-E-cadherin (Immamura et al., 1999; Nagafuchi et al., 1994; Ozawa and Kemler, 1998), z-catenin-vinculin (Ozawa and Kemler, 1998; Watabe-Uchida et al., 1998), and z-catenin-formin-1 (Kobielak et al., 2004), all of which partially rescue cell-cell adhesion. However, no direct evidence was presented in those studies that these chimeric proteins bound actin filaments. Moreover, our findings that the molecular and functional properties of z-catenin are altered upon binding to p-catenin—in particular, that the z-catenin/p-catenin complex cannot bind actin—demonstrate that the use of E-cadherin/z-catenin chimeras cannot recapitulate the behavior of the cadherin-catenin complex at the membrane. It is possible that expression of some of these chimeric proteins could locally change actin dynamics or simply increase the amount of cadherin at the cell surface to a level that can partially rescue cell-cell adhesion.

Although it is surprising that a stable linkage does not exist between cadherins and the underlying actin cytoskeleton, cell-cell adhesion is a dynamic process during embryonic development, wound healing, and cancer cell metastasis (Takeichi, 1995; Thiery, 2002). This may require a more dynamic interaction between cadherin and the actin cytoskeleton rather than the static, stable linkage proposed in previous models. In addition, it is noteworthy that, in most cell types, cadherins are not the only means of cell-cell adhesion. Many other adhesion proteins are expressed, including members of the nectin occludin/claudin, JAM, and desmosomal cadherin families (Gestios et al., 2004; Takai and Nakaniishi, 2003), all of which are thought to interact directly or indirectly with the actin or intermediate filament cytoskeletons and thereby contribute to cell-cell adhesion.

There are dramatic changes in membrane and actin dynamics associated with the formation of cell-cell adhesions. Initial cell-cell contact formation is driven by overlapping membrane lamellipodia from contacting cells. These lamellipodia are regulated by actin polymerization and branching induced by the Arp2/3 complex (Kovacs et al., 2002) and local activation of the Rho family of small GTPases (Braga, 2002; Fukata and Kaibuchi, 2001). However, lamellipodial activity decays as the cadherin-catenin complex accumulates and the contacts mature into stable cell-cell junctions (Ehrlich et al., 2002; Vaezi et al., 2002). It is not known what regulates this contact-dependent decrease of membrane activity, which presumably depends upon a decrease in Arp2/3-mediated actin polymerization. It is interesting to note, in this context, that decreased levels of z-catenin result in increased membrane activity in hippocampal neurons, while overexpression of z-catenin suppresses membrane activity (Abe et al., 2004), indicating that z-catenin directly regulates membrane protrusive activity. Furthermore, keratinocytes from z-catenin knockout mice are characterized by loss of contact inhibition and increased migratory activity (Vasioukhin et al., 2001).

How might changes in both actin assembly and organization (from networks to bundles; see Hirokawa et al., 1983) that drive the transition from active lamellipodia to quiescent contacts (see Ehrlich et al., 2002) be coordinated during the formation of cell-cell adhesions? Our finding that z-catenin suppresses Arp23-mediated actin polymerization in a concentration-dependent manner, combined with the actin bundling activity of z-catenin (Rimm et al., 1995), may provide an explanation (Figure 7). The cytoplasmic z-catenin monomer concentration (0.6 μM) is too low to bind actin significantly and would need to be concentrated to bind actin and to dimerize. A significant increase in the local concentration of z-catenin at the membrane occurs upon accumulation of the cadherin-catenin complex at nascent contacts. This cadherin bound pool of z-catenin can exchange with the cytoplasmic pool (Figure 4); note that we probably underestimate the amount of exchange because we cannot directly measure it locally at cell-cell contacts. Although the local concentrations of z-catenin and Arp23 immediately adjacent to contacting membranes are unknown, a 10-fold increase in local concentration of z-catenin would be sufficient for z-catenin to compete with the Arp23 complex for actin filaments (Figure 7). This would suppress formation of branched actin networks and inhibit lamellipodial activity and would also favor formation of z-catenin homodimers that bundle actin filaments (Rimm et al., 1995), resulting in a reorganization of actin filaments and a change in membrane dynamics underneath the junction (Figure 7). It has also been proposed that formins, which promote formation of linear actin cables, are recruited to the adherens junction by z-catenin (Kobielak et al., 2004). If so, z-catenin would serve as a switch that turns off Arp23-mediated branched-actin-network formation required for lamellipodial activity during the initiation of adhesion and turn on z-catenin-mediated bundling of actin filaments and formation of linear cables by formins during maturation of the adherens junction. While further work is needed to test specific tenets of this hypothesis, our results provide new mechanistic insights into many aspects of the local dynamics of actin and...
membranes associated with cell-cell contacts not accounted for in previous models.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification

E-cadherin cytoplasmic domain, β-catenin, plakoglobin, α-catenin, and the β-catenin-α-catenin chimera were expressed and purified as described in the accompanying paper (Yamada et al., 2005).

Chromatography

Analytical size exclusion chromatography was done in 20 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM DTT on a Superdex 200 column. Proteins were injected at a 25.6 mM concentration. For binding studies, proteins were mixed and incubated overnight at 4ºC.

Multiangle Light Scattering

α-catenin monomer and β-catenin were analyzed on a Superdex 200 column attached to a UV detector followed by a multiangle light scattering (MALS) DAWN EOS (Wyatt Technology, Santa Barbara) and a refractive index (RI) detector. The system was equilibrated with a buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM DTT, and the response of the light scattering detector was normalized by measuring the signal of monomeric bovine serum albumin (BSA). A value of 0.185 ml/g was assumed for the dn/dc of BSA and α-catenin. Scattering intensities measured at nine different angles (detectors 7–15) were used for data analysis. The molecular weight of the protein was calculated with the ASTRA IV program using the signal from the MALS and the RI detector.

Limited Proteolysis

Full-length α-catenin monomer and dimer and β-catenin were analyzed on a Superdex 200 column attached to a UV detector followed by a multiangle light scattering (MALS) DAWN EOS (Wyatt Technology, Santa Barbara) and a refractive index (RI) detector. The system was equilibrated with a buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM DTT, and the response of the light scattering detector was normalized by measuring the signal of monomeric bovine serum albumin (BSA). A value of 0.185 ml/g was assumed for the dn/dc of BSA and α-catenin. Scattering intensities measured at nine different angles (detectors 7–15) were used for data analysis. The molecular weight of the protein was calculated with the ASTRA IV program using the signal from the MALS and the RI detector.

Microscopy and Image Analysis

Methods are described in the accompanying paper (Yamada et al., 2005).

Supplemental Data

Supplemental Data include one figure and one movie and can be found with this article online at http://www.cell.com/cgi/content/full/123/5/903/DC1/.

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Figure 7. Model of α-Catenin Function in Regulating Actin Dynamics and Organization

Initial cell-cell contact is mediated by interactions of cadherins present on the membranes of lamellipodia. Clustering of cadherins at the nascent contacts leads to accumulation of cadherin-catenin complexes. A high local concentration of α-catenin is produced when it dissociates from these complexes. α-catenin, which exists as monomer or homodimer, competes with Arp2/3 complex for actin filaments (the dimer more potently than the monomer), thereby suppressing Arp2/3-mediated actin assembly that drives lamellipodia. α-catenin also bundles actin filaments, which may contribute to the reorganization of actin in the mature contact.
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