Structure and Mechanism of Cadherins and Catenins in Cell-Cell Contacts

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Abstract

Cadherins are Ca\textsuperscript{2+}-dependent cell adhesion molecules found in several kinds of cell-cell contact, including adherens junctions and desmosomes. In the presence of Ca\textsuperscript{2+}, cells expressing the same type of cadherin form stable contacts with one another, a phenomenon designated homophilic, or homotypic, adhesion. Most cadherins are single-pass transmembrane proteins whose extracellular regions mediate specific cell-cell interactions. The intracellular faces of these contacts are associated with the actin cytoskeleton in adherens junctions or the intermediate-filament system in desmosomes. The close coordination of the transmembrane adhesion molecules with the cytoskeleton is believed to be essential in coordinating morphogenetic movements of tissues during development and in conferring the appropriate mechanical properties to cell-cell contacts. Structural, biochemical, and biophysical analysis of the molecules that comprise these contacts has provided unique mechanistic insights into the specificity of homophilic adhesion, the functional connection to the underlying cytoskeleton, and the dynamics of junction formation.

Key Words

adherens junction, desmosome, β-catenin, α-catenin, homophilic adhesion
INTRODUCTION

Cadherins are Ca^{2+}-dependent cell adhesion molecules found in vertebrates as well as invertebrates (Cox & Hardin 2004, Nollet et al. 2000). Cadherins have a fundamental role in cell recognition during embryogenesis (Gumbiner 2005, Takeichi 1995, Tepass 1999), during which specific expression patterns mark specific cell types and tissues. Virtually all cells that form solid tissues express cadherins, and cadherin expression is tightly regulated in the developing as well as in the adult organism. Downregulation or loss of cadherins in tumor cells often correlates with a more invasive phenotype (Gumbiner 2005, Hajra & Fearon 2002).

Cell contacts initiated by the cadherin ectodomains strengthen over a time scale of minutes owing to rearrangements of the cytoskeleton near the developing junction (Chu et al. 2004). Moreover, during development adhesion must be closely linked to cell-shape changes that enable morphogenetic movements as tissues remodel. Thus, the cytoplasmic side of cadherin-based cell junctions has an essential role in cell contact formation and in the interplay between adhesion and development.

This review focuses on structural, biophysical, and biochemical studies of cadherin-based adhesion. These investigations have provided important mechanistic insights into the specificity of cell-cell adhesion and its interplay with the cytoskeleton.

CADHERIN ECTODOMAIN INTERACTIONS

Homophilic and Heterophilic Cadherin Interactions

Initial experiments demonstrated that cells expressing different subtypes of cadherins segregate when mixed (Nose et al. 1988), and switches in the expression of particular cadherins correlate with the formation of specific tissues during embryonic development (Gumbiner 2005). These observations led to the concept that cadherins function...
as homotypic cell adhesion molecules. However, the experimental methods used to assess specificity, which include the monitoring of cell aggregation after transfection by particular cadherins, binding of cells to immobilized cadherin ectodomains, or binding of beads coated with purified cadherins, have given rise to somewhat conflicting results. In some cases, only those cells expressing the same kind of cadherin adhere in aggregation or sorting assays (Inuzuka et al. 1991, Murphy-Erdosh et al. 1995, Nose et al. 1988, Patel et al. 2006, Shan et al. 2000, Shimoyama et al. 2000), whereas other studies show considerable promiscuity in cadherin interactions (Duguay et al. 2003, Niessen & Gumbiner 2002, Patel et al. 2006, Shimoyama et al. 2000, Volk et al. 1987).

In addition to the particular types of cadherins expressed on the cell surface, the levels of expression and shear force to which the cells are subjected during mixing influence the degree of homo- or heterophilic aggregation and sorting (Chu et al. 2004, Duguay et al. 2003, Nose et al. 1988, Steinberg & Takeichi 1994). For example, mixtures of cell lines expressing significantly different levels of the same cadherin segregate, whereas a closer match of surface cadherin levels can produce mixed cell aggregates as opposed to sorting into discrete clusters of cells bearing a single subtype. These findings suggest that adhesive specificity arises from both affinity differences between cadherin subtypes and their expression levels (Foty & Steinberg 2005). Moreover, certain pairs of cadherins can cross-adhere at low shear forces but will segregate at higher shear forces (Duguay et al. 2003). Because cells remain in contact longer at lower shear force than at higher forces, the kinetics of cadherin-cadherin interactions may have a role in determining cell specificity. Overall, it appears that at the molecular level homophilic specificity is not strict, at least within the major subclasses of cadherins, and that small differences in binding energy can give rise to specificity differences. The molecular basis of this concept is discussed below.

### Overall Structure of Cadherins

Several groups have determined the three-dimensional structures of type I cadherin ectodomain fragments, including N-cadherin EC1 (Shapiro et al. 1995a), N-cadherin EC1–2 (Tamura et al. 1998), E-cadherin EC1 (Overduin et al. 1995), E-cadherin EC1–2 (Haussinger et al. 2004, Nagar et al. 1996, Pertz et al. 1999), and the complete C-cadherin ectodomain EC1–5 (Boggon et al. 2002). Patel et al. (2006) recently reported several type II cadherin fragment crystal structures, including cadherin-11 EC1–2, cadherin-8 EC1–3, and MN-cadherin EC1. The overall dimensions of the EC domain are 45 Å by 25 Å by 25 Å. The EC structure is composed of seven β-strands arranged as two opposing β-sheets with N and C termini at the opposite ends (Figure 1a). Successive strands are labeled A–G; strands A, G, F, and C form one sheet, and strands D, E, and B form the other sheet. This Greek-key topology is identical to that of immunoglobulin domains, but conserved packing interactions that stabilize the fold are distinct in the two superfamilies (Shapiro et al. 1995b). The five EC domains within the type I and type II cadherins have very similar structures (Boggon et al. 2002, Patel et al. 2006).

Ca++ binding and Ca++ dependence of cadherin-based adhesion define this family of adhesion molecules. Ca++ rigidifies the cadherin ectodomain, as seen by loss of sensitivity to proteolysis, direct visualization by electron microscopy, and analysis of the relative motions of successive domains by nuclear magnetic resonance (NMR) spectroscopy (Haussinger et al. 2002, Pokutta et al. 1994). Crystal structures reveal that three Ca++ bind between successive domains and are coordinated by conserved amino acids at the base of one domain and the top of the next (Figure 1a,b). The coordination scheme is very similar in each of the four interdomain interfaces. Binding to the shared Ca++ produces a well-defined tilt between successive domains, which gives the full cadherin...

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**Homophilic (homotypic) adhesion:** intercellular contact of cells expressing the same cadherin

**Heterophilic (heterotypic) adhesion:** intercellular contact of cells expressing different cadherins

**NMR spectroscopy:** nuclear magnetic resonance spectroscopy
(a) Domain structure, Ca\(^{2+}\) coordination, and strand-exchange dimer of C-cadherin (Boggon et al. 2002). Cadherin domains form a seven-stranded \(\beta\)-sandwich structure. \(\beta\)-strands of the first domain are labeled A to G: Strands C, F, G, and A (red) form one sheet, and strands D, E, and B (yellow) form the other sheet, of the \(\beta\)-sandwich. Three Ca\(^{2+}\) bind between successive domains. Ca\(^{2+}\) and Ca\(^{2+}\)-coordinating residues are shown in green. The strand-exchange interaction between two molecules is shown; the second molecule is colored in blue, and the Trp2 residues of each molecule are in yellow. (b) Three-dimensional structure of the entire C-cadherin extracellular domain. N- and O-linked carbohydrates visible in the structure are shown in cyan. (c) Strand-exchange interface of C-cadherin, colored as in panel a. Figure prepared with PyMol (DeLano 2002).

Tomography: a microscopy method in which a series of two-dimensional images of a sample is obtained in different orientations, then computationally merged to generate a three-dimensional image.

The structure of the full C-cadherin ectodomain expressed in Chinese hamster ovary cells also revealed 3 N-linked and 12 O-linked glycosylation sites in domains 2–5, with all but 2 sites in domains 3 and 4 (domains 2 and 5 have one O- and one N-linked site) (Boggon et al. 2002) (Figure 1b). The O-linked sites on the G strand of EC3 and EC4 appear to be well conserved, but their biological significance is unclear.

Molecular Basis of Cadherin Specificity

A number of electron microscopy studies implicate the outermost (EC1) domains in cadherin-cadherin interactions. First, thin sections of adherens junctions show rods with thickness and length corresponding to cadherins that project off the membrane. Prominent density at the midline between membranes is most simply interpreted as overlaps of the ends of the molecules. The intermembrane distance is approximately 25 nm (Miyaguchi 2000), and the molecules appear to project at an angle rather than perpendicular to the membrane. Analysis of cryopreserved, fully hydrated desmosomes reveals a prominent midline density likely due to EC1-EC1 interactions, albeit at a larger intermembrane spacing of 33 nm (Al-Amoudi et al. 2004, 2005). A three-dimensional tomographic reconstruction of desmosomes prepared by freeze substitution and plastic embedding shows low-resolution electron density that agrees well with the C-cadherin
ectodomain structure (He et al. 2003). Modeling of the C-cadherin ectodomain structure into this density shows only EC1-EC1 contacts, with an intermembrane spacing of 28 nm. A three-dimensional cryo-electron microscopy reconstruction of VE-cadherin EC1–4 reconstituted on liposomes also reveals a curved ectodomain conformation, and the ectodomains from opposed membranes contact each other via EC1-EC1 interactions (Lambert et al. 2005). Although variation of the intermembrane spacing likely arises from differences in tissue and sample preparation—staining and embedding apparently result in smaller distances than do fully hydrated specimens (Al-Amoudi et al. 2004)—the consistent picture is one indicating that outer EC1 domains interact.

Cells expressing chimeric E- and P-cadherins in which one or more of the EC domains are swapped display adhesive specificity of the N-terminal EC domain (EC1) (Nose et al. 1990). Similar experiments showed that EC1 dictates the segregation of cells expressing type I and type II cadherins (Patel et al. 2006). An in vivo assay of motor neuron pool sorting demonstrated that the fates of cells expressing chimeric type II cadherins were determined by the identity of the EC1 domain (Patel et al. 2006). These data offer compelling evidence that EC1 is the major determinant of cadherin specificity.

Structural data have provided considerable insight into both cadherin specificity and cross-reactivity mediated by the EC1 domain. In crystal structures of the N-cadherin EC1 (Shapiro et al. 1995a), E-cadherin EC1–2 (Haussinger et al. 2004), C-cadherin EC1–5 (Boggon et al. 2002), and three type II cadherins (Patel et al. 2006), two EC1 domains associate intimately by exchange of their N-terminal β-strands (Figure 1a,c). The conserved Trp2 is buried in a hydrophobic pocket of the partner domain, and flanking residues form specific interactions to produce a strand-swapped or strand-exchanged dimer. In the type II cadherins, a second conserved Trp at position 4 also binds to a pocket on the partner. The repeated observation of the strand-swapped dimer between EC1 domains suggests that the dimer is a key aspect of cadherin-cadherin interactions.

Among type I cadherins, the strand-exchange interaction varies both in the amount of surface area buried in the interface as well as the relative orientation of the two EC1 domains. For example, the relative orientation of the two EC1 domains in C-cadherin differs by 25° from that seen in the N-cadherin EC1 dimer. In contrast, the EC1-EC1 interaction of type II cadherins features a substantially larger interaction surface, owing largely to an extended region of nonpolar contacts that runs along the entire length of the interface (Patel et al. 2006). The larger interface appears to constrain the inter-EC1 angle, which varies among the three known type II structures by only 6°. The differences in the nature of the strand-exchange contacts likely explain the absence of cross-reactivity between type I and type II cadherins (Patel et al. 2006). In contrast, the first seven amino acids of β-strand A have at the most two amino acid substitutions in type I cadherins, with Trp2, Pro4, and Pro5 invariant, and a similar level of conservation exists in this region of type II cadherins. The conserved nature of these interfaces likely allows heterotypic interactions within a subclass, at least under some experimental conditions.

Mutational data support the importance of the strand-exchange interface in cadherin-cadherin interactions. Mutation of Trp2 to Ala or mutations in the pocket that binds to Trp2 abolish adhesion of type I cadherins (Kitagawa et al. 2000, Shan et al. 2000, Tamura et al. 1998), and mutation of either Trp2 or Trp4 abolishes adhesion in a type II cadherin (May et al. 2005). The introduction of cysteine residues at positions that would form a disulfide bond in a strand-swapped dimer produces cross-linked molecules in extracts of such cells (Harrison et al. 2005a).

NMR measurements of E-cadherin EC1–2 showed that at low protein concentrations Trp2 binds into the pocket of the same strand-exchange mode of cadherin dimerization in which a β-strand from one cadherin domain binds to another domain and vice versa.
cadherin molecule, suggesting that this arrangement represents the monomeric structure of E-cadherin (Haussinger et al. 2004). At higher concentrations this construct forms the strand-exchanged dimer. Thus, strand exchange occurs in solution and is highly dynamic. The dynamics of strand exchange are substantially altered when extra residues are present at the N terminus, consistent with the observation that removal of a prodomain is required to achieve cell adhesiveness (Ozawa & Kemler 1990). The native N terminus forms a salt bridge with Glu89, an interaction conserved in classical cadherins (Patel et al. 2006), and mutation of the acidic residue or extension of the N terminus (Harrison et al. 2005b, Kitagawa et al. 2000, Ozawa & Kemler 1990) prevents adhesion. Interestingly, β-strand A has been found to be bound to a partner molecule or to the same molecule or, alternatively, to be disordered (i.e., exposed and not bound to the rest of the protein) in crystal structures of cadherins with or without extensions of the N terminus, indicating that crystallization conditions also influence the equilibrium (Nagar et al. 1996, Overduin et al. 1995, Pertz et al. 1999, Shapiro et al. 1995a, Tamura et al. 1998).

Cadherin interactions depend on the presence of Ca$^{2+}$. Disruption of Ca$^{2+}$ coordination in the EC1-EC2 interface abolishes the strand-exchange interaction (Chitaev & Troyanovsky 1998). NMR analysis revealed distinct conformations for the Ca$^{2+}$-free monomer, Ca$^{2+}$-bound monomer, and Ca$^{2+}$-bound dimer of E-cadherin EC1–2 (Haussinger et al. 2004). Ca$^{2+}$ binding shifts the equilibrium toward the dimeric state; the dissociation constant, $K_D$, for dimerization changes from 10 mM in the absence of Ca$^{2+}$ to 0.7 mM in the presence of Ca$^{2+}$ (Haussinger et al. 2004). Comparison of the Ca$^{2+}$-bound E-cadherin monomer and strand-exchanged dimer structures reveals that the largest change in backbone torsion angles occurs at residues 14 to 16. These residues are located in the loop that connects the A and B β-strands. This loop includes Glu11 and Asn12, which directly coordinate Ca$^{2+}$ at the EC1-EC2 interface. Thus, Ca$^{2+}$ binding at the base of the A strand influences the strand-exchange equilibrium.

The on rate for dimerization proves to be several orders of magnitude lower than that of a diffusion-limited process, indicating that dimerization is strongly hindered by an activation barrier (Haussinger et al. 2004). This is intuitively reasonable given that to dimerize, interactions between β-strand A and residues in the hydrophobic pocket have to be broken and reestablished and that several hydrophobic residues are exposed in the intermediate state.

In domain-swapping interactions such as the strand exchange between EC1 domains, the monomeric form of the protein acts as a competitive inhibitor to dimer formation and thereby lowers the binding affinity (Liu & Eisenberg 2002). Chen et al. (2005) have noted that the low-affinity interaction produced by strand exchange makes it possible to achieve specific cell sorting by closely related cadherins. In a simple binding equilibrium, if the concentration of the reactants is lower than the $K_D$, few molecules of product will be present. In this regime, small differences in intrinsic affinity produce significant differences in the number of adhesive dimers formed and hence in the total adhesive energy (i.e., the product of the intrinsic free energy of dimer formation and the number of dimers).

Initial cell-cell contacts are made by cadherins present on the surface of highly dynamic lamellipodia. If the membranes stay in contact for a sufficient length of time, more cadherin molecules can diffuse into the contacting region, and the adhesive interaction strengthens owing to multivalency (Chen et al. 2005). A weaker contact with smaller numbers of cadherin heterodimers is more likely to dissociate before the interaction strengthens. Thus, multiple weak interactions can produce specific homotypic cell-cell interactions. Intrinsically strong cadherin-cadherin interactions, in contrast, would likely have slow dissociation rates that
would maintain inappropriate heterotypic interactions long enough for the contact to strengthen. Measurements of cadherin interactions by single-molecule methods reveal short- and longer-lived bonds, and the shorter-lived state may be important for initial exploratory processes before the establishment of a stable contact (Perret et al. 2004). There are differences in the off rates of E- and C-cadherin, which may indicate that intrinsic kinetic differences contribute to cadherin-based cell sorting (Bayas et al. 2006).

**Potential Lateral (cis) Interactions of Cadherins from the Same Membrane**

In the first crystal structure of the N-cadherin EC1 domain, packing interactions observed in several crystal forms suggested two kinds of cadherin-cadherin interactions (Shapiro et al. 1995a). The first was the strand-exchanged dimer, which features a parallel arrangement of domains. At the time this was interpreted as representing lateral *cis* interactions of cadherins emanating from the same membrane. A second interaction with an antiparallel orientation of EC1 was interpreted as the *trans* interaction between cadherins from opposing cell surfaces. Mutagenesis experiments indicate that the latter interactions are not relevant to biological function (Kitagawa et al. 2000, Klingelhofer et al. 2002). The subsequent C-cadherin ectodomain structure shows that the highly curved conformation can give rise to the parallel, strand-swapped interaction of EC1 domains from cadherins on opposing surfaces (Figure 2a), and the tomographic desmosome reconstruction also

![Figure 2](image-url)

(a) Strand-exchange dimer as seen in the crystal structure of the C-cadherin full-length extracellular region. In panels a and b, protomers are colored in red and blue, and Ca\(^{2+}\) are shown as small green spheres. (b) Packing in the C-cadherin ectodomain crystals (Boggon et al. 2002). (c) Schematic drawing of the proposed contacts formed between cadherin molecules (orange) in surface force measurements (Sivasankar et al. 2001). Panels a and b were prepared with PyMol (DeLano 2002).
indicates that this interaction represents the adhesive trans configuration (He et al. 2003).

Other potential cis interactions have been suggested on the basis of crystallographic packing interactions. Perhaps the most intriguing is that from the five-domain C-cadherin ectodomain structure, in which EC1 of one molecule contacts EC2 of another; this interaction is also present in several two-domain structures of E-cadherin (Boggon et al. 2002, Haussinger et al. 2004, Nagar et al. 1996, Pertz et al. 1999). However, this interaction has not been seen in N-cadherin (Tamura et al. 1998), and NMR studies of E-cadherin EC1–2 (Haussinger et al. 2004) have not provided evidence for cis interactions involving these surfaces. Nonetheless, mutation of R-cadherin Asp44, which in C-cadherin lies near the base of EC1 and is near the EC2-EC3 interface, diminishes adhesiveness (Kitagawa et al. 2000), suggesting that these interactions may be relevant. If so, it will be important to examine more constructs with intact Ca2+ sites at the EC2-EC3 interface because disrupted Ca2+ sites can affect adhesion.

Engel and coworkers have generated artificial dimers, trimers, and pentamers of cadherins by fusion to the fos-jun dimer (E- and P-cadherins) (Ahrens et al. 2002), the trimerization domain of cartilage matrix protein (VE-cadherin) (Ahrens et al. 2003), or the pentamerization domain of cartilage oligomeric protein (COMP) (E-cadherin) (Pertz et al. 1999, Tomschy et al. 1996). Electron microscopic images revealed rings that are most simply interpreted as the association of two cadherin molecules in cis through their N-terminal EC1 domains (Figure 3). Adhesive trans interactions between pentamers were observed only between ring structures, suggesting that the lateral cis dimer is a functionally adhesive unit (Figure 3). The images in Figure 3 provide independent confirmation that the outermost domains mediate trans interactions.

The lateral dimers associate with other lateral dimers at unexpectedly low concentrations of COMP–E-cadherin of 0.1–0.6 μM (Tomschy et al. 1996). In contrast, no association of monomeric cadherin ectodomains was observed under the same conditions, consistent with the 0.7-mM K_D for homoassociation of the EC1–2 constructs measured by NMR (Haussinger et al. 2004). These data suggest that lateral dimerization may enable rapid formation of a second trans interaction, effectively creating a multivalent interaction with twice the free energy of a single interaction.

To determine the interfaces involved in the trans and cis dimers, cysteines were introduced into EC1 positions that would be cross-linked by a thiol-specific cross-linker in the strand-swapped dimer. When confluent cells grown in the presence of Ca2+ were subjected to this treatment, the cadherins were cross-linked (Troyanovsky et al. 2003). In
addition, cross-linking also occurred in extracts of dissociated, noncontacting cells in the absence of Ca$^{2+}$. These data indicate that lateral dimers can form on the same surface in the absence of Ca$^{2+}$ by similar strand-exchange interactions that appear important for the trans adhesive interaction. cis interactions apparently can also form in the presence of Ca$^{2+}$ because the formation of rings within the pentameric E-cadherin–COMP fusion proteins requires Ca$^{2+}$ concentrations of 0.5–1.0 mM. The W2A mutation in the E-cadherin-COMP chimera abolished trans interactions between pentamers but did not affect cis association within the pentamer (Pertz et al. 1999). This result may suggest an interface distinct from that involved in strand exchange. However, another explanation is that the W2A mutation weakens the association of $\beta$-strand A with the partner molecule sufficiently to ablate the detection of trans interactions but that, at high local concentrations within the pentamer, the interaction can still occur. The occurrence of lateral strand-exchanged dimers was also suggested by the tomographic reconstruction of desmosomes, which showed cadherin molecules from the same cell surface contacting each other at their N-terminal domains (He et al. 2003).

Role of the Inner EC Domains

The inner EC domains are essential for cadherin function. C-cadherin–Fc fusion proteins that have one or more EC domains deleted were examined in cell attachment and bead-binding assays (Chappuis-Flament et al. 2001). The EC1–2 protein did not mediate adhesion as efficiently as did longer constructs, and the insertion of fibronectin repeats between the cadherin EC1–2 and Fc domains to increase spacing from the membrane and/or conformational flexibility did not increase the affinity. However, the addition of either EC3 or EC4 restored adhesive activity. In contrast, a construct consisting of EC3–5 fused to Fc did not display adhesive activity. Thus, cadherin domains following EC2 contribute to the homophilic interaction but do not by themselves support specific adhesion. The requirement for a third EC domain may reflect the need for an intact Ca$^{2+}$ site at the base of EC2.

Leckband and colleagues have used a surface force apparatus (SFA) to examine the interaction between cadherins on opposed surfaces (Prakasam et al. 2006a; Sivasankar et al. 1999, 2001; Zhu et al. 2003). Purified cadherins bearing C-terminal affinity tags are attached to lipid bilayers that have been deposited on opposed mica surfaces. Both the force and distance between the surfaces can be measured as the surfaces are moved together or apart. Given the geometry of the surface and the density of cadherins, an estimate of the bond energy per molecule can be obtained. When the surfaces are retracted, bond rupture causes a loss in the attractive force, resulting in a jump in the intersurface distance. After one accounts for linker lengths, the positions of these jumps represent the distance at which cadherins on opposed surfaces interact. Several force minima were found for E-, C-, and N-cadherin (Prakasam et al. 2006b; Sivasankar et al. 1999, 2001).

Sivasankar et al. (2001) interpreted force jumps at discrete distances on the basis of the length of a single cadherin domain and the assumption that the molecules are more-or-less perpendicular to the membrane. The C-cadherin ectodomain structure shows that if two such opposed molecules had their EC1 domains interacting through the strand-exchange interface, the total intermembrane distance would be 385 Å (Figure 2a). This distance corresponds to the “outer” distance seen in the force measurements when linkers are taken into account. An EC1-EC3 contact would explain the intermediate distance of 320 Å, and almost complete interdigitation would explain the inner bond of 250 Å (Figure 2c). Interactions involving inner domains may explain why cells expressing an E-cadherin mutant lacking EC1 do not aggregate but can form mixed aggregates with cells expressing full-length E-cadherin.
suggesting interactions between EC1 and other cadherin domains (Renaud-Young & Gallin 2002).

Zhu et al. (2003) tested the interdigitation model, using C-cadherin constructs with different domain deletions. Fc fusion constructs lacking EC5, or EC4 and EC5, show force minima at three discrete membrane separations that are shifted relative to the full five-domain molecule by distances corresponding to the loss of the EC domains from the opposed molecules. Curiously, the strength of the interactions, including that of the outermost interaction ascribed to EC1-EC1 contacts, is reduced significantly in the deletion constructs. A construct lacking the first two domains (C-cadherin EC345) adhered weakly to itself, or to EC1–5 or EC1–3, at distances corresponding to interactions between EC3 domains of two opposing molecules. The latter result is not entirely consistent with bead-binding and cell attachment assays, which indicated that EC3 is not required for adhesive interactions (Chappuis-Flament et al. 2001).

The correlation of distance with the number of domains assumes that the domains are arranged in a more-or-less linear end-to-end fashion. However, crystal structures of C-cadherin EC1–5 and cadherin-8 EC1–3, as well as electron microscopic images of E-cadherin, desmosomal cadherins, and VE-cadherin, indicate that the molecules have a pronounced curvature (Boggon et al. 2002, He et al. 2003, Lambert et al. 2005, Patel et al. 2006, Pokutta et al. 1994). In the full five-domain C-cadherin structure, the long axis of EC1 is almost perpendicular to that of EC5 (Figure 1b). The intermembrane space corresponding to an EC1-EC1 interaction would be expected somewhere in the 385-Å range if these molecules are oriented perpendicular to the membrane, but tilting the molecules from the membrane would reduce this distance. For example, in the C-cadherin EC1–5 crystal lattice, contacts between EC1 domains from opposed layers of the lattice occur at a spacing of 245 Å (Figure 1e) (Boggon et al. 2002).

The interdigitated domain model is difficult to reconcile with several electron microscopy studies of native or artificial cadherin-based contacts, all of which indicate that only the EC1 domains interact (see above) and that they do so at distances shorter than the 385-Å outer bond ascribed to EC1-EC1 interactions. Moreover, as noted above, when cysteines are introduced into positions that would be cross-linked in the strand-exchange EC1-EC1 dimer, cadherins extracted from confluent cells that have been exposed to the cross-linker are dimerized (Troyanovsky et al. 2003). Because the cross-linker is added to confluent, adhesive monolayers, it would appear that the strand-swapped interaction occurs at adhesive equilibrium and does not represent an intermediate state.

Despite the lack of structural evidence for interactions involving the inner EC domains, it is difficult to explain why the inner bond is lost when EC1245 is used in the force experiments or why EC345 shows an interaction. The loss of the outer bond in the W2A mutant (Prakasam et al. 2006a) is also consistent with a model involving interactions between inner domains. In this case, the inner bond becomes substantially weaker, and this may be due to a long-range allosteric effect of the EC1-domain mutation. However, mutations in the hydrophobic core of EC2 do not affect cell adhesion (Kitagawa et al. 2000), which seems to argue against the idea that long-range changes are transmitted through this domain.

Given the structural and biochemical evidence for EC1-EC1 interactions and an absence of such data for other pairings, can the SFA data be explained without resort to other domain contacts? The EC1-EC1 interaction can occur at the observed distance of 250 Å simply by having an angled orientation with respect to the membrane (Figure 2b). This is reasonable, given that the molecules are
attached to the lipid surface via a flexible linker, which would allow bending at the base. In an atomic force microscopy (AFM) study, VE-cadherin-Fc fusion proteins were attached to a surface and an AFM tip with extensible linkers. Unbinding forces of 40, 80, and 120 pN were observed, suggesting multivalent binding of a 40-pN quantum; the longer the tip and surface were in contact, the more 120-pN interactions were observed (Baumgartner et al. 2000). This was ascribed to the formation of lateral interactions that produce multivalent binding interactions. As noted above, crystal packing interactions have suggested the existence of lateral contacts involving inner domains. A recent electron microscopy study of VE-cadherin suggests that the inner EC domains are required to form lateral associations important for multivalency (Hewat et al. 2007).

Extending this notion, the lateral association of inner domains may orient the cadherin molecules such that the EC1s are displayed at a fixed distance from the membrane in a way that maximizes the number of trans-interacting molecules. A larger number of interacting molecules at the inner distance is consistent with the higher bond energies observed, and the fixed orientation would explain why the observed interactions rupture at a discrete distance. The higher valency may also explain why a weak interaction of the W2A mutant can be observed at the inner distance: Although the tryptophan side chain is not available to fill the pocket, the other interface contacts may still form, producing a weaker interaction that is detectable when enough molecules are involved.

Ultimately, methods that can directly relate the SFA and other force measurements to structural and biochemical data on cadherin ectodomain interactions will be required to resolve the apparent conflict between these sets of experiments. At the same time, more structural data from intact ectodomains may help to determine the nature of potential lateral or interdigitated adhesive arrangements involving the inner EC domains.

**INTRACELLULAR PROTEIN INTERACTIONS: THE CADHERIN-CATENIN COMPLEX**

The intracellular side of cadherin-based contacts contains multiple proteins that functionally link cadherins to the underlying cytoskeleton. In adherens junctions, the cytoplasmic region of cadherin binds to β-catenin (or its close relative plakoglobin) and to p120. β-Catenin in turn binds to α-catenin. α-Catenin has a number of binding partners, including actin. On the basis of these binary interaction data, it was assumed for many years that α-catenin mediates a stable link between the cadherin–β-catenin complex and actin. Recent experiments have challenged this view and have suggested alternative roles for α-catenin in the junction.

**β-Catenin and p120**

β-Catenin is a highly conserved 781-amino-acid protein whose primary structure includes an N-terminal region of approximately 150 amino acids, a central ~520-residue domain composed of 12 armadillo (arm) repeats, and a C-terminal 100-residue region. E-cadherin binds to the arm domain, whereas α-catenin binds to residues 118–149, just before the start of the arm domain. Each arm repeat has three α helices, designated H1, H2, and H3; repeat 7 lacks H1. H2 and H3 are antiparallel to one another and pack against the flanking repeats to form an elongated superhelical structure (Huber et al. 1997) (Figure 4a). The superhelix features a groove whose floor is composed of H3 and whose walls have contributions from H1 and the loop that connects H2 and H3. This groove proves to be the binding site for E-cadherin and other β-catenin ligands involved in Wnt signaling (see Choi et al. 2006 and references therein). The portion of the groove formed by repeats 5–9 binds to the extended peptide motif Dxθφx₂⁻θ-E, where θ is an aliphatic and φ an aromatic amino acid, which is found in cadherins and many other...
β-catenin–E-cadherin complex

There are few structural data for the N- and C-terminal regions that flank the arm repeats. The proteolytic sensitivity of β-catenin indicates that these regions are flexibly linked to the arm domain. Although there have been reports that these regions influence the binding of ligands (Castano et al. 2002, Gottardi & Gumbiner 2004, Piedra et al. 2001), direct measurement of the affinities of full-length, arm only, N+arm, or arm+C β-catenin constructs for various ligands showed no significant differences for cadherins, the transcription factor Lef-1, or the transcriptional inhibitor ICAT (Choi et al. 2006). However, the tails influence the binding of weaker ligands APC and axin, although the mechanism for this is not understood (Choi et al. 2006).

p120 is an arm-family protein whose arm repeat domain binds to the juxtamembrane region of the cadherin cytoplasmic domain (Daniel & Reynolds 1995, Ohkubo & Ozawa 1999, Thoreson et al. 2000). Although its role is unclear, p120 may locally control regulators of the actin cytoskeleton and the rate of cadherin endocytosis (Anastasiadis & Reynolds 2001, Davis et al. 2003). The structure of the arm domain of the desmosomal p120 homolog plakophilin 1 has been solved (Choi & Weis 2006). Although sequence alignments had suggested that the p120 subfamily contains ten arm repeats, the structure reveals only nine repeats; one of the predicted repeats in the middle of the protein forms a large insertion (Figure 4b). The ends of this insertion form a wedge that introduces a pronounced kink into the domain, making plakophilin 1 quite distinct from β-catenin (Figure 4). Nonetheless, the superhelical structure of the domain gives rise to a prominent groove. The significance of the groove has not yet been investigated by mutational or structural methods.
Cadherin Cytoplasmic Domain

The cytoplasmic domain of classical cadherins, ~150 amino acids, is the most highly conserved region of these molecules (Nollet et al. 1999). The cytoplasmic domains of desmosomal cadherins have regions homologous to that of the classical cadherins, although the desmoglein cytoplasmic domain is considerably larger. In the absence of β-catenin, the cytoplasmic domain is unstructured (Huber et al. 2001) and is an example of an intrinsically unstructured protein (Dyson & Wright 2005, Tompa 2005). Crystal structures of the full 151-residue E-cadherin domain bound to β-catenin show that the last 100 residues become ordered in the complex (Huber & Weis 2001) (Figure 4a). The first 51 residues contain the p120-binding site, and some part of this region likely becomes ordered upon the formation of that complex.

The lack of structure of E-cadherin in the absence of β-catenin is consistent with several aspects of cadherin biology. Newly synthesized E-cadherin associates with β-catenin while still in the endoplasmic reticulum, and the two proteins move together to the cell surface (Hinck et al. 1994). Disruption of this interaction leads to proteosomal destruction of cadherin (Chen et al. 1999). The E-cadherin sequence features a PEST motif (characterized by the presence of Pro, Glu or Asp, and Ser or Thr flanked by basic residues), which is recognized by ubiquitin ligases. The PEST motif is present in the β-catenin-binding region of cadherin and would be inaccessible in the complex with β-catenin (Huber & Weis 2001) (Figure 4a, region 4). This modification increases the on rate and decreases the off rate of the interaction (Choi et al. 2006), and the region that becomes structured overlaps the PEST region (Huber & Weis 2001). Thus, one role of phosphorylation may be to further reduce the likelihood that cadherin will be degraded.

Phosphorylation of β-catenin Tyr654 (repeat 12) by src kinase also modulates the interaction of β-catenin with E-cadherin, but in the opposite direction. In the absence of phosphorylation, the phenolic hydroxyl group of the tyrosine side chain forms a hydrogen bond with an aspartate from cadherin region 2 (Figure 4a) (Huber & Weis 2001). Phosphorylation disrupts this interaction and results in a roughly sixfold diminution in affinity (Roura et al. 1999). Again, the significance of these changes is not known, but they are correlated with a change in cell contacts and the development of a more invasive cell, consistent with dysregulation of the junctional complex.

The phosphorylation data highlight an important aspect of the unstructured cadherin tail. Because the binding interface is based essentially on a linear peptide sequence rather than a preformed three-dimensional structure, the interaction is somewhat modular. That is, a given stretch of cadherin sequence can interact with β-catenin, whereas flanking regions remain disordered. Four crystallographically independent views of the molecule (two with unphosphorylated and two with phosphorylated E-cadherin) show that certain
regions are always bound, whereas others are bound in only some of the copies (Figure 4a).

For example, regions 2 and 3 are seen in all copies, whereas region 5 is seen in three of four copies. Region 4 is seen only when E-cadherin is phosphorylated; presumably the unstructured nature of this region when not bound makes it a good substrate for kinases. Thus, the unstructured nature of the ligand allows for the modulation of affinity by phosphorylation without complete dissociation of the complex. This likely introduces a subtle level of control that allows graded responses to cellular conditions beyond a simple on-off switch mechanism.

α-Catenin

α-Catenin is a 906-amino-acid protein that is homologous to the focal adhesion protein vinculin. Many α-catenin binding partners have been identified (Kobielak & Fuchs 2004), although not all have been verified biochemically with pure proteins. Limited proteolysis and sequence homology to vinculin have shown that the protein is composed of distinct domains. There are three major regions defined in this manner, as outlined in the following subsections.

Dimerization and β-catenin binding. Purified recombinant α-catenin forms a homodimer, that, upon binding to β-catenin, dissociates to form a 1:1 heterodimer (Koslov et al. 1997, Pokutta & Weis 2000). The dimerization/β-catenin-binding region lies near the N terminus of the protein, in residues 57–264 (Pokutta & Weis 2000). The structure of the dimerization domain defined by proteolysis, residues 82–264, starts as a 30-residue α helix, followed by an antiparallel 50-residue helix and then three more helices (Figure 5a) (Pokutta & Weis 2000). The first half of the long helix pairs with the first helix, and the second half forms part of an antiparallel four-helix bundle at the C terminus of the domain. The two antiparallel N-terminal helices pair with the corresponding helices in the other protomer to form a noncovalent, antiparallel four-helix bundle (Figure 5a).

Alanine scanning mutagenesis demonstrated that β-catenin residues 118–149 bind to α-catenin largely as an α helix (Aberle et al. 1996). α-Catenin residues 57–81, which limited proteolysis indicates are flexibly linked to the dimerization domain, are also predicted to form an α helix (Pokutta & Weis 2000). On the basis of these observations, we proposed a model for the interaction in which the α-catenin-binding site of β-catenin and α-catenin residues 57–79 form two helices that replace one protomer of the α-catenin homodimer to generate a β-catenin–α-catenin heterodimer (Figure 5a) (Pokutta & Weis 2000). This implies that the binding of α-catenin to β-catenin competes with homodimerization, which has important implications for α-catenin function, as described below.

To test this model, Pokutta & Weis (2000) produced a chimeric protein, designated βα-catenin, in which the α-catenin-binding site of β-catenin was fused to residues 57–264 of α-catenin by a flexible linker. This strategy generated a high local concentration of β-catenin that outcompetes the homodimerization interaction to create a stable mimic of the β-catenin–α-catenin interaction. The crystal structure of this chimera (Figure 5a) has two unexpected features (Pokutta & Weis 2000). First, the packing of the β-catenin and α-catenin 57–79 helices onto the other two α-catenin helices differs from the homodimer packing and features a slightly larger interface. This may account for the observation that when the two proteins are mixed, the β-α heterodimer is favored (Koslov et al. 1997). Second, the β-catenin helix is interrupted at residue 141; residues 142–144 are bound in an extended conformation. β-Catenin Tyr142 is buried in the interface. This tyrosine is a substrate for Met kinase, and phosphorylation of this residue diminishes the β-α interaction (Brembeck et al. 2004).

Gel filtration of MDCK cell extracts revealed that the bulk of endogenous α-catenin
Figure 5

(a) The three-dimensional structures of the α-catenin dimerization region and a βα-catenin chimeric protein lead to a model of α-catenin–β-catenin interaction (Pokutta & Weis 2000). The protomers of the α-catenin dimer are shown in yellow and orange. The helix indicated by the hatched blue line represents residues 59–82, which form a helix in βα-catenin (right) but are not present in the structure of the dimerization domain (left). In the βα-catenin chimera helices of the dimerization region are colored in yellow, and the helix N-terminal to it is shown in blue. The α-catenin-binding region of β-catenin is shown in red. A schematic diagram of the βα-catenin construct is shown below the structure. Figure prepared with PyMol (DeLano 2002).

(b) Schematic diagram of the βα-catenin full-length construct.

is monomeric (Drees et al. 2005). Small amounts of both α-catenin homodimer and β-catenin–α-catenin heterodimer were also detected. Using Western blots calibrated with known amounts of recombinant α-catenin, Drees et al. (2005) estimated the cytosolic concentration of α-catenin to be approximately 0.6 μM. Mixtures of α-catenin monomer and dimer can be found in purified recombinant material soon after purification, when applied to a gel filtration column at a concentration of 2 μM. These observations suggest that the K_D for α-catenin homodimer formation lies in the low-μM range. Gel filtration experiments performed with reasonably pure monomer suggested that the
$K_D$ for $\beta$-catenin binding is roughly 1 $\mu$M (Drees et al. 2005), similar to the estimate obtained from surface plasmon resonance measurements (Koslov et al. 1997).

**M domain.** A second, proteolytically defined region in the middle of the $\alpha$-catenin sequence, designated the M domain, spans residues 377–633 (Pokutta et al. 2002, Yang et al. 2001). The M domain is composed of two tandem four-helix bundles connected by a short linker (Figure 6a). Two different crystal structures, each with several crystallographically independent copies in the crystal, have shown that the two subdomains can adopt a wide range of relative angles, varying up to 56° (Pokutta & Weis 2002, Yang et al. 2001). The interdomain flexibility may be important for transmitting conformational changes between the N-terminal $\beta$-catenin-binding and C-terminal actin-binding regions, as discussed below.

The M domain is the binding site for the protein afadin (Pokutta et al. 2002), an actin-binding protein associated with the Ig-superfamily adhesion molecule nectin. The biology of this adhesion system is poorly understood, but this system is thought to be involved in the early establishment of cell contacts (Takai & Nakanishi 2003). Both M subdomains are required for the interaction (Pokutta et al. 2002), which implies that afadin binds to a particular arrangement of the two domains.

**Actin-binding domain.** Both $\alpha$-catenin and vinculin bind to filamentous actin. The highest level of sequence homology between these two proteins is found in their C-terminal actin-binding domains (Bakolitsa et al. 1999, Pokutta et al. 2002, Rimm et al. 1995). The actin-binding domain of vinculin is a five-helix bundle (Bakolitsa et al. 1999) (Figure 6b), and a recent cryo-electron microscopy study of this domain bound to F-actin identified a putative interaction surface (Janssen et al. 2006). Sequence alignments indicate that the five-helix architecture and the actin-binding surface residues are conserved in $\alpha$-catenin (Bakolitsa et al. 1999). Surprisingly, however, a fragment of $\alpha$-catenin spanning the homology region (corresponding to the crystal structure of the vinculin domain) does not bind to actin (Pokutta et al. 2002). A 42-amino-acid sequence unique to $\alpha$-catenin that extends past the homology region to the C terminus is also required for actin binding, indicating that there are important differences in how vinculin and $\alpha$-catenin recognize actin (Pokutta et al. 2002).

**Role of $\alpha$-Catenin in Junctions and a Model for Cell-Cell Contact Formation**

The discovery of the ability of $\alpha$-catenin to bind to both $\beta$-catenin and F-actin led to the conclusion that $\alpha$-catenin links the cadherin–$\beta$-catenin complex to actin, providing a stable mechanical linkage between the extracellular contact and the underlying cytoskeleton. Recent work, however, has shown that this notion does not stand up to experimental scrutiny (Yamada et al. 2005). Experiments with purified recombinant proteins showed that $\alpha$-catenin cannot bind to $\beta$-catenin and actin simultaneously, even in the presence of
of native membranes, cytosol, or other proteins reported to bind to both actin and α-catenin. The dynamics of these proteins were also examined in living cells. In fluorescence recovery after photobleaching experiments, E-cadherin, β-catenin, and α-catenin displayed very similar diffusional behaviors on the membrane, as would be expected if they had formed a complex, whereas actin associated with cell-cell contacts diffused more rapidly and was more mobile (Yamada et al. 2005). Moreover, deletion of the cadherin cytoplasmic domain or the actin-binding domain of α-catenin, either of which would break the putative link to actin, did not significantly alter the dynamics of the cadherin-catenin complex (Yamada et al. 2005). These results independently confirmed the lack of a stable linkage between the cadherin-catenin complex and actin at cell-cell contacts.

At the molecular level, different conformational states of α-catenin appear to be associated with β-catenin or actin binding. The α-catenin homodimer competes with β-catenin binding, which may explain why the α-catenin monomer preferentially binds β-catenin. In contrast, the α-catenin homodimer binds to actin more strongly than does the monomer, and the monomer may not be able to bind to actin at all (Drees et al. 2005). Proteolytic sensitivity experiments indicate that the α-catenin monomer, the α-catenin homodimer, and the α-catenin–β-catenin heterodimer possess distinct molecular conformations (Drees et al. 2005). Thus, it appears that α-catenin acts as an allosteric protein in which binding to one partner negatively regulates the affinity for the other. Conformational changes are likely transmitted between the N-terminal β-catenin-binding domain and the C-terminal actin-binding domain; the observed flexibility in the M domain structures is consistent with such a model.

The finding that α-catenin does not mediate a stable linkage between cadherin and actin seems to contradict data from cadherin–α-catenin fusion proteins that bypass β-catenin and can restore adhesion to cells (Imamura et al. 1999, Nagafuchi et al. 1994, Sako et al. 1998). The physiological relevance of the chimeras is questionable given that β-catenin fundamentally changes the actin-binding activity of α-catenin. Moreover, the behavior of cells expressing the chimeras can be explained without the invoking of a stable linkage to actin (Weis & Nelson 2006).

If α-catenin does not serve as a link to generate a stable cadherin-catenin-actin complex, what is its role in cell contact formation? The formation of cadherin-based adhesions begins with transient contacts between lamellipodia bearing cadherin on their membranes. Lamellipodia are dynamic structures formed by Arp2/3-stimulated polymerization of branched actin networks that push the membrane outward (Pollard & Borisy 2003). As the contact matures, membrane dynamics decrease, and active lamellipodia are replaced by a more uniform, stable membrane. This transition is accompanied by changes in the underlying actin cytoskeleton (Adams & Nelson 1998, Ehrlich et al. 2002, Vaezi et al. 2002), which becomes organized into linear cables (Hirokawa et al. 1983).

Several lines of evidence indicate that α-catenin regulates actin assembly and dynamics in cell-cell contacts. The reduction in or overexpression of α-catenin in dendritic spines produces enhanced or decreased membrane activity, respectively (Abe et al. 2004). Moreover, the deletion of α-catenin results in increased cell migration, a shortened cell cycle, and increased proliferation rather than simple loss of cell-cell adhesion (Lien et al. 2006, Vasioukhin et al. 2001). α-Catenin can suppress Arp2/3 activity by competing with Arp2/3 for actin filaments (Drees et al. 2005). Complete suppression occurs at an α-catenin concentration of 5 μM. However, little suppression occurs when the concentration of α-catenin is 0.5 μM, roughly its bulk cytosolic concentration (Drees et al. 2005).

The effect of α-catenin on Arp2/3 has been rationalized in a model that explains many of the properties of α-catenin in developing cell-cell contacts (Figure 7).
Initial contacts between lamellipodia lead to further engagement of the membranes and the clustering of cadherins. Because cell-surface cadherins are bound to β-catenin, the clustering of cadherins creates a high local concentration of β-catenin at the contact site. β-Catenin-bound α-catenin readily exchanges with cytosolic α-catenin (Drees et al. 2005). The binding of α-catenin monomers to the clustered cadherin–β-catenin complex, followed by dissociation, creates a high local concentration of α-catenin that can dimerize. The resulting population of α-catenin dimers near the membrane is at a high enough concentration to suppress Arp2/3, resulting in a cessation of lamellipodial movement to make a more stable contact. The ability of α-catenin to bundle actin (Rimm et al. 1995) may also have a role in reorganizing actin into the linear bundles observed in mature contacts. Moreover, α-catenin may positively regulate formin-1, which promotes the formation of linear actin cables (Kobielak et al. 2004).

Thus, α-catenin may actively mediate the switch between Arp2/3-stimulated branched actin polymerization and the formation of formin-stimulated linear actin cables.

Actin is associated with mature cell junctions, but its role may vary, depending on the status of the tissue. To the extent that adherens junctions provide strength to tissues, it may suffice to correctly organize actin into a gel with the appropriate mechanical properties. However, morphogenetic changes during tissue development require the close coordination of cell adhesion and actomyosin-mediated cell-shape change, and there is likely a direct linkage between junctional components and actomyosin under these circumstances (Dawes-Hoang et al. 2005, Young et al. 1991). What might link actin to cell junctions during active constriction? Nectins are another class of adhesion molecules found in adherens junctions (Takai & Nakanishi 2003). The cytoplasmic domain of nectins binds to afadin, which is an actin- and

Figure 7
Model of α-catenin function in actin polymerization and reorganization in developing adherens junctions. Initial contacts between cadherins on opposed lamellipodia lead to cadherin clustering. α-Catenin bound to the clustered E-cadherin–β-catenin complex rapidly exchanges between β-catenin and the cytosol, thereby producing a locally high concentration of α-catenin that can dimerize and suppress the activity of Arp2/3.
α-catenin-binding protein (Pokutta et al. 2002, Takai & Nakanishi 2003). However, a direct connection between nectins and actin has not been demonstrated with purified proteins. Another candidate is Shroom, a PDZ domain–containing, actin-binding protein required for neural tube formation found in the adherens junction (Hildebrand 2005). An interesting possibility is Vezatin, a transmembrane protein located in adherens junctions that links the cadherin-catenin complex to myosin (Kussel-Andermann et al. 2000). Finally, the synaptotagmin-like protein Bitesize binds to moesin, an actin-binding protein. Bitesize mutants do not properly organize actin at the adherens junction during Drosophila cellularization (Pilot et al. 2006). Whether these or other proteins are needed for the coordination of cell-cell contacts with actomyosin-based cell-shape change during development awaits further experimentation.

**SUMMARY POINTS**

1. Cadherins are Ca\(^{2+}\)-dependent cell adhesion molecules. The cadherin extracellular region (ectodomain) mediates cell-cell contact. The ectodomain contains tandem repeats of characteristic extracellular cadherin (EC) domains that are linked by Ca\(^{2+}\). Cadherin specificity is encoded by the N-terminal EC1 domains, which interact through the exchange of N-terminal β-strands. Strand exchange involves binding of a conserved Trp residue into the hydrophobic core of the partner and the formation of other contacts around this site.

2. The inner EC domains are required for adhesion, but their roles remain controversial. One model proposes that the specificity-determining interaction between EC1s is weak and represents an intermediate state and that a strong adhesive bond is formed by the interdigitation of ectodomains involving contacts between inner domains. The other model postulates that the inner domains are needed for lateral associations of cadherins on the membrane that produce multivalent interactions required for strong adhesion.

3. The intracellular side of cell-cell contacts formed by classical cadherins is composed of the cadherin intracellular domain bound to β-catenin, which in turn binds to α-catenin. α-Catenin binds to actin, and it had been thought that this interaction led to direct and stable linkage of classical cadherins to actin. However, recent data indicate that α-catenin binds to β-catenin and actin in a mutually exclusive manner; a stable assembly of the cadherin-catenin complex is not found either biochemically or in cell-imaging experiments.

4. Initial cell-cell contacts form between highly dynamic lamellipodia on the surface of nonadherent cells that are driven by Arp2/3-stimulated polymerization of branched actin networks. α-Catenin can suppress polymerization stimulated by the Arp2/3 complex. The concentration at which this suppression occurs is approximately 10 times higher than the bulk cytosolic concentration of α-catenin. On the basis of these observations, it was proposed that cadherin clustering arising from the interaction of cadherin ectodomains produces an increase in the local concentration of the cadherin–β-catenin–α-catenin complex. Dissociation of α-catenin from these complexes produces a locally high concentration of α-catenin sufficient to suppress Arp2/3 and therefore membrane dynamics, leading to the formation of a stable contact between cells.
DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED


The only crystal structure of a complete cadherin ectodomain. Strand-exchange interactions in the crystals show that this mode of interaction can result from cadherins on opposed surfaces owing to the curvature of the molecule.

Shows that the oligomeric state of α-catenin modulates its binding to β-catenin and to actin and that α-catenin can suppress the activity of the Arp2/3 complex.
Proposes a model for α-catenin as a regulator of actin organization in developing cell-cell contacts.
Provides a detailed characterization of strand exchange in solution, including the effects of N-terminal processing and Ca\(^{2+}\) binding on the strand-exchange equilibrium.

Details the structural basis of the E-cadherin cytoplasmic domain–\(\beta\)-catenin interaction, including the effects of phosphorylation.


Renaud-Young M, Gallin WJ. 2002. In the first extracellular domain of E-cadherin, heterophilic interactions, but not the conserved His-Ala-Val motif, are required for adhesion. *J. Biol. Chem.* 277:39609–16


SFA measurements of the cadherin ectodomain interactions show interactions at discrete distances that are interpreted as interdigitation of domains.

Defines the mechanisms of α-catenin homodimerization and binding to β-catenin.


Demonstrates that α-catenin cannot bind simultaneously to β-catenin and to actin and that there is, therefore, no stable link between the cadherin–β-catenin complex and actin.
The cadherins comprise a family of single-pass transmembrane proteins critical for cell–cell adhesion in vertebrates and invertebrates. The recently determined structure of the whole ectodomain from C-cadherin suggests that the adhesion of cadherins presented by juxtaposed cells is mediated by a strand-swapped dimer in which core hydrophobic elements are exchanged between the partner molecules. Sequence analysis suggests that several cadherin subfamilies share this adhesive mechanism. Recent work has shed new light on the molecular basis of cadherin adhesion, although understanding the specificity of these interactions remains a major challenge.

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**Abbreviations**

EC extracellular cadherin domain

EM electron microscopy

**Introduction**

The cadherins comprise a family of calcium-dependent cell adhesion molecules that form and maintain adhesive contacts between the cells of solid tissues [1–3]. Many events in the development of complex tissue structures are associated with changes in cadherin expression [4,5,6]. This has led to the prevailing view that selective cell adhesion mediated by cadherins provides a key driving force in the development of tissue architecture [6–8].

Recent studies, however, indicate that interaction between cadherins can be more promiscuous [26,27,28,29]. Although homophilic binding still appears to be preferred, many type I cadherins can form productive cell adhesive interactions with other type I cadherins. Some type I cadherins (such as N-cadherin and E-cadherin) induce the formation of separate cell aggregates when transfectants are mixed, whereas others (such as N-cadherin and R-cadherin) will co-aggregate. The single in-depth study on the adhesive specificity of type II cadherins yields similar results for this subfamily [27]. Despite the cross-reactivity of cadherins within the same subfamily, it appears that the binding specificities of type I and
type II cadherins are orthogonal. That is to say, type I cadherins may have graded affinities for other type I cadherins and type II cadherins have graded affinities for others of their class \[28^\text{C15}\]. However, current data suggest that type I and type II cadherins do not interact. The ‘orthogonality’ of the type I and type II cadherin adhesion systems may have important consequences for how these molecules function in tissues where many different cadherins may be expressed \[5^\text{C15}\].

In development, cadherins can function in the formation of cell layers (e.g. the separation of the neural tube from the ectoderm, mediated by N-cadherin and E-cadherin) and also in the formation of highly complex tissue structures. Figure 2 shows the expression patterns of four different cadherins in the developing mouse retina, visualized by in situ hybridization \[4\]. Various stages of development are shown, illustrating the extraordinary temporal and spatial regulation of classical cadherins.
The recent crystal structure of the whole ectodomain (EC1–EC5) from type I C-cadherin [13**] revealed a pair of molecules in a symmetric dimer formed through the interaction of the partner EC1 domains (Figure 4a). The interface is mediated by the exchange of the N-terminal β-strands between the partner EC1 domains. A central feature is the insertion of the conserved Trp2 sidechain from one molecule into the hydrophobic core of the other (Figure 4e). This interface, called a cadherin ‘strand dimer’, had been observed before; structures of EC1 from the classical cadherin N-cadherin revealed an identical configuration [9].

This strand-dimer interface plays a critical role in cadherin-mediated cell adhesion and we are of the opinion that it is the primary adhesive interface of cadherins. Several lines of evidence support this idea. First, W2 and the residues that line its acceptor pocket are highly conserved among cadherins. Second, cadherin-mediated aggregation is abolished (for all cadherins tested thus far) by mutation of this tryptophan to alanine (W2A, Figure 3f,g) or by mutation of one of the alanine residues of the acceptor pocket to encode a larger residue, methionine (A80M, Figure 3h). Such a residue would be expected to block tryptophan insertion [24]. Furthermore, a zebrafish lethal developmental mutation, glass onion, has been shown to encode a W2G mutant of N-cadherin [34*]. Finally, the tryptophan sidechain analog indole-3-acetic acid has been shown to function as an inhibitor of cadherin adhesion, both in cell-based experiments [24] and in experiments with purified protein [35**]. The inability of the related compound 5-methyl indole 3-acetic acid, which modeling studies suggest

**Figure 2**

Illustration of cadherin function in the developing mouse retina. Complex spatial and temporal regulation of cadherin expression is thought to guide the formation of tissue architecture through the differential cell adhesive specificity of different cadherins. The panels show in situ hybridizations for the type I molecules N-cadherin and R-cadherin, and the type II molecules cadherin-8 and cadherin-11, at postnatal days 0, 7 and 21. The classical cadherins are found in the zonula adherens in contacts between adjacent cells. Note that each cadherin adopts markedly different expression patterns and these change over time during retinal development. Small arrows indicate amacrine cells, large arrows indicate horizontal cells and arrowheads show bipolar cells. The following abbreviations are used to indicate the cell layers of the retina: GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; and OLM, outer limiting membrane. Adapted from [4].

the formation of this complex structure, which consists of multiple cell types arranged in several different cell layers. In light of the large variety of cadherins expressed in such tissues, it seems likely that heterophilic as well as homophilic interactions will be formed.

Despite the importance to development and tissue homeostasis of selective cell adhesion mediated by cadherins, the atomic-level basis of their specificity is still not understood. In this review, we focus on recent high-resolution structural results and discuss their implications for understanding the cell adhesive interactions of cadherins.

**Structural basis of homophillic adhesion**

Type I and type II cadherins each contain five tandem cadherin repeat domains in their extracellular region (EC1–EC5). A key question is to define which of these repeats harbors the cadherin–cadherin binding and specificity sites. This question has been addressed, in one set of experiments, through the study of the behavior of chimeric molecules in cell aggregation assays (Figure 3) [23,26]. In summary, N-cadherin and E-cadherin transfectants sort to form separate tissue-like cell aggregates, but swapping domains between N-cadherin and E-cadherin can sometimes swap the specificity of cell aggregation. Notably, it is the N-terminal, membrane-distal domain of each molecule (EC1) that carries this property. These experiments provide strong evidence supporting the idea that the adhesive binding site of cadherins is localized primarily within the EC1 domain (see also Update). Additionally, cadherins are synthesized as pro-proteins with a pre-domain at the N terminus of EC1; this must be removed to activate adhesive function [30]. The proximity of this small pre-domain to EC1 provides additional support for the notion that binding and specificity are dominated by interactions involving the EC1 domain and/or regions close to it. Further evidence comes from the work of Engel and collaborators [31,32,33*], who have reported a variety of creative EM studies of cadherin fusion proteins, which have uniformly shown interaction between cadherins at or near their N-terminal domains.
Cadherins may be able to dimerize in *cis* orientations (between molecules presented on the same cell), as well as *trans* (molecules from different cells). Interestingly, biochemical studies employing precipitation of epitope-tagged cadherins from transfected cells indicate that both *cis* and *trans* dimers do indeed form, and that they arise through the use of the same interface residues [26,36,37]. Although this may seem, at first, counterintuitive, the long somewhat flexible nature of the five-domain cadherin extracellular region appears to put little constraint on this alternative usage, which may play an important role in the biology of cadherins. Specifically, it is possible that cadherins may exist as *cis* strand dimers on a single cell surface, and that these *cis* dimers may be replaced by or exist in equilibrium with *trans* strand dimers in the presence of a juxtaposed cadherin-presenting cell.

Cadherins mediating cell adhesion must bind together from two opposing cells. Thus, the adhesive bond must form at the cell surface between cadherins that have been synthesized and processed in different cells. It is not then surprising that monomeric forms of cadherins should exist and indeed have been observed in high-resolution structural studies [10,12,24,32]. These monomeric forms reveal properties of the adhesive interface that are informative about potential mechanisms of adhesion. Two monomer forms of EC1 have been observed: a structure with the A-strand (which encompasses W2) disordered and the W2 acceptor pocket exposed to solvent (Figure 4b,c); and the A-strand closed form (Figure 4d), in which W2 inserts into its own acceptor pocket rather than that of a partner protomer. This plasticity of the A-strand suggests unique properties that are likely to be important for strand-dimer formation between cadherins presented by juxtaposed cells. Two crystal structures and an E-cadherin EC1 NMR structure reveal that the A-strand, which is required to complete the β-sandwich fold of EC1, is disordered. As a result, conserved hydrophobic residues are exposed to solvent. This conformational heterogeneity suggests intrinsic instability of the A-strand in the monomer, as it is disordered in some structures and bound in others. The absence of a stable structure should
favor the ejection of the critical W2-containing A-strand from the protomer body and is likely to be a key element driving the formation of strand-dimer interactions. It should be noted, as a caveat, that some of the monomeric cadherin structures were obtained from cadherin fragments that contained extra amino acids at the N terminus as the result of cloning artifacts. This could be significant, as cell-based studies have shown that inexact cleavage of the pre-domain (leaving as few as four amino acids extra at the N terminus) can abolish the adhesive function of E-cadherin [30]. The kinetics of association of individual E-cadherin fragments have been studied by flow chamber analysis [35**]. Association of these purified proteins was shown to be dependent on W2 and inhibited by indole 3-acetic acid. Furthermore, the bond duration was remarkably brief, estimated at approximately 2 s. Although the full implications of this rapid exchange are not yet clear for cadherin function at the cellular level, this observation may provide a rationale for the apparent need for the clustering of cadherins at cell-cell contacts [38,39].

Although we think that the majority of data indicate that EC1 is the primary site of adhesive binding in cadherins, surface force experiments [40,41] and some cell-based assays [42] suggest the involvement of other domains. In the surface force studies, the extracellular portions of C-cadherin molecules are bound to two opposing plates through C-terminal 6-His tags. These plates are then brought together and the force required to separate them is measured as a function of the interplate distance. The results of these experiments revealed force maxima at several discrete distances. This led the investigators to suggest that different cadherin repeats contribute directly to adhesion through multiple binding sites that form distinct interdigitated conformations to produce complexes of different lengths. In a similar vein, cell aggregation...
experiments with membrane-proximally truncated extra-
cellular regions of C-cadherin [42] (EC1–EC4, EC1–EC3 etc.) show that the EC1–EC3 construct mediates strong
adhesion, but EC1–EC2 is only weakly adhesive and EC1
not at all. For N-cadherin, the EC1–EC2 truncated form
can mediate strong adhesion (DR Colman, W Shan,
personal communication). The molecular basis of the
requirement for at least two domains in cell aggregation
assays is not evident from the crystallographic studies.
One possibility is that additional domains are required for
a minimum clearance from the cell surface, which of
course contains myriad other proteins. Another possibility
involves a potential active role for Ca\textsuperscript{2+} ligation (which
requires more than the EC1 domain) in inducing a con-
formational change to an adhesion-competent state. It is
of interest in this regard that an NMR study revealed
chemical shift changes in residues lining the W2 acceptor
pocket upon Ca\textsuperscript{2+} ligation, even though these residues
are at least 20 Å away from the Ca\textsuperscript{2+}-binding sites [10,43].
This explanation does not, however, explain the surface
force experiments. A third possibility is that other mole-
cular interfaces, yet to be identified, are involved in
adhesion. The primary difficulty with this idea comes
from the domain-swap experiments, which indicate that
binding specificity is localized exclusively to EC1.

**Potential specificity determinants**

Multiple sequence alignment of EC1 domains from
representative cadherins (Figure 5) illustrates the highly
conserved nature of W2 and residues lining its hydro-
phobic acceptor pocket. The protocadherins do not share
this conservation, suggesting the possibility that they may
use a different interface to mediate adhesion. Nonethe-
less, the strand dimer probably represents a binding mode
that is common to type I, type II and desmosomal
cadherins. Although peripheral residues may change

![Figure 5](image-url)

Multiscip sequence alignment and pairwise sequence identities of EC1 regions of representative cadherin superfamily members. (a) Highlighted in
green are the conserved Ca\textsuperscript{2+}-binding sites, which provide the most recognizable sequence determinants of cadherin sequence repeats. Red
highlighted positions indicate conserved tryptophan residues: W2 constitutes a central element of the adhesive interface in type I cadherins and
W4, which is conserved in type II cadherins, may be involved in adhesion in this cadherin subclass. Residues in magenta line the hydrophobic pocket
that accommodates the conserved W2 in the classical cadherins. Highlighted in gray are analogous hydrophobic pocket positions for type II and
desmosomal cadherins, inferred from sequence analysis. The blue highlighted position shows a conserved glutamic acid residue that is involved in
forming a salt bridge with the N terminus in the adhesive interface of type I cadherins. Above the alignment are the β-strand positions determined
by crystallographic analysis of C-cadherin. (b) Within each cadherin subfamily, there is high sequence identity. Pairwise sequence alignments were
obtained using CLUSTALW 1.75 with default gap penalty parameters and the BLOSUM series of scoring matrices. Pcdh, protocadherin.
during evolution to provide binding specificity, the major elements of the cadherin–cadherin interface remain unchanged. Thus, cadherins appear to be different from other classes of adhesion molecules, such as those of the immunoglobulin superfamily [44], which display many structurally disparate modes of interaction.

The conservation of the central elements of the adhesive interface provides a simple explanation for the cross-reactivity of binding among subfamily members. On the other hand, there must be substantial differences between the binding interfaces of type I and type II cadherins, as they do not bind to one another. Indeed, type II cadherins show distinct differences from canonical type I sequences. Most notably, as is evident in Figure 5a, type II cadherins have two conserved tryptophan residues in their A-strands (W2 and W4), rather than a single conserved tryptophan. Additional potentially significant differences between type I and type II cadherins are suggested by crystal structures of type I cadherins. For example, the sidechain at position 23 of type I cadherins is partially buried upon strand-dimer formation and is involved in an intermolecular hydrogen bond with the sidechain at position 8; these residues are always polar in type I cadherins. However, most type II cadherins have phenylalanine at position 8 and a positively charged residue at position 23, so that a comparable hydrogen-bonding interaction is not possible. Understanding the significance of these differences must await the determination of crystal structures of type II cadherins.

Possible specificity determinants for cadherins within a single subfamily (i.e. type I to type I interactions) include those residues present in the adhesive interface, excluding the conserved W2 and small hydrophobic pocket residues, which are common to all subfamily members. Currently, structures of dimeric conformations are available only for C-cadherins and N-cadherins [9,13**]. With this limited set of structures, it is difficult to extract principles that could explain specificity among type I cadherins generally, which show graded levels of affinity for one another.

Conclusions

Cadherins appear to function in cell adhesion through a common mechanism centered around β-strand exchange between monomers and the insertion of hydrophobic residues (W2 in the case of type I cadherins) into the core of the partner molecule. This mechanism of interaction appears to be common to cadherins known to function in cell adhesion and specificity has probably arisen through changes in residues peripheral to these central interface elements. Cadherin EC1 domains have unique properties that destabilize ‘closed’ conformations and promote the partial disassembly of the domain (by ejection of the A-strand), thus enabling strand-dimer formation. The human genome contains about a hundred cadherin genes and it seems likely that those that function in intercellular adhesion do so through this common mechanism. Evidence for this can be seen in the amino acid sequences of type I, type II and desmosomal cadherins, but not the protocadherins. Thus, structural analysis of protocadherins is a high priority in order to gain a better understanding of the cadherin adhesive repertoire.

Our current understanding of cadherin specificity is poor at best. Although we can now be reasonably confident about the identity of the interface that mediates cadherin adhesion, little is known about the factors determining the binding preferences of cadherins. Additional structural studies will undoubtedly shed light on this issue, but these will need to be augmented by a variety of computational tools. There has been considerable progress in our understanding of the energetic basis of protein–protein interactions [45] and it will be of interest to exploit what has been learned to elucidate the design principles used by cadherins to achieve specificity. Some of the questions that need to be addressed for cadherins are quite general, as it is not uncommon for protein–protein recognition modules to share common affinity-determining elements while achieving specificity in other regions of the interface.

Update

Using electron tomographic reconstructions of plastic sections of neonatal mouse skin, He et al. [48**] visualized the organization of desmosomes in situ at resolutions corresponding to between ~12 and ~30 Å. The crystal structure of the ectodomain from C-cadherin could be modeled into the tomographic density with good fit. This study clearly shows that the predominant site of interaction between the desmosomal cadherin molecules is the EC1 domain. Furthermore, this study presents evidence for the presence of both cis and trans dimers mediated through the EC1 domain, consistent with previous structural and biochemical analyses of the strand dimer.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


Cadmherin-mediated cell–cell adhesion Patel et al. 697


This paper compares the expression patterns of type II cadherins in the developing spinal cord of chick embryos. The authors demonstrate, for the first time, extracellular markers of different motor neuron pools, which have previously only been delineated by the expression patterns of transcription factors. They also show that cadherins can drive the segregation of neurons into different motor pools by the ectopic expression of MN-cadherin in the chick embryo.


The first high-resolution crystal structure of the whole functional ectodomain of a type I cadherin, C-cadherin, is presented. The structure shows an adhesive interface involving the N-terminal cadherin domains of two cadherin protomers. This structure supports the strand-dimer model of the binding interaction between cadherin molecules.


Before the publication of this paper, there had been several conflicting reports as to whether or not the proto-cadherin gene cluster was regulated by trans splicing. These authors show, through a number of beautiful experiments, that intracluster splicing occurs via a conventional cis splicing mechanism.


The authors performed adhesion flow assays using purified cadherin proteins and cells expressing cadherins. They observed that none of the cadherin-expressing cells exhibited substantial adhesive specificity for either of the two purified cadherin proteins tested in the assay. This study indicates that classical cadherins may be far more promiscuous in their binding interactions with other classical cadherins than was previously thought.


This EM study of the complete ectodomain of a type II cadherin fused to the oligomerization domain of cartilage matrix protein reveals Ca2+ dependent ring-like and double ring-like arrangements involving the EC1 cadherin domains. Similar ring-like structures were observed in earlier analogous EM studies of type I cadherins. This suggests a common mechanism of adhesion for type I and type II cadherins.

34. Malicki J, Jo H, Pujic Z: Zebrafish N-cadherin, encoded by the glass onion locus, plays an essential role in retinal patterning. Dev Biol 2003, 259:95-108. The authors use genetic screens to show that the glo1/+ allele encodes a W2G substitution in the N-cadherin gene. This mutant allele, which is embryonically lethal, causes severe disruption of neural architecture. This paper indicates the critical role of the W2 amino acid in the EC1 domain of N-cadherin.

35. Perret E, Benoile AM, Nassy P, Pierres A, Delmas V, Thiery JP, Bongrand P, Feracci H: Fast dissociation kinetics between individual E-cadherin fragments revealed by flow chamber analysis. EMBO J 2002, 21:2537-2546. This is the first reported study to provide quantitative data on the time-scale of the kinetics of the binding interaction between cadherins, using an in vitro flow chamber. Glass beads coated with cadherin EC1-EC2...
fragments were sent through a laminar flow chamber also coated with cadherin EC1–EC2 fragments. A camera was used to monitor the progress of a glass bead; binding timescales were calculated based upon how long a bead was retarded in the chamber. The unstressed life-time of individual E-cadherin interactions was as brief as 2 s.

36. Klingelhofer J, Laur OY, Troyanovsky RB, Troyanovsky SM: Dynamic interplay between adhesive and lateral E-cadherin dimers. Mol Cell Biol 2002, 22:7449-7458. This study employed a mixed culture co-immunoprecipitation assay involving cells expressing E-cadherin tagged with either Myc or Flag epitopes. Ca²⁺-binding site mutants of E-cadherin were constructed, and cells expressing these mutants can form both adhesive (trans) dimers and lateral (cis) dimers. A W2A mutant formed neither type of dimer. This suggests that the interface mediating cis and trans dimers may be one and the same.


