The cadherin–catenin complex as a focal point of cell adhesion and signalling: new insights from three-dimensional structures

Jane M. Gooding, Kyoko L. Yap, and Mitsuhiko Ikura*

Summary

Cadherins are a large family of single-pass transmembrane proteins principally involved in Ca^{2+}-dependent homotypic cell adhesion. The cadherin molecules comprise three domains, the intracellular domain, the transmembrane domain and the extracellular domain, and form large complexes with a vast array of binding partners (including cadherin molecules of the same type in homophilic interactions and cellular protein catenins), orchestrating biologically essential extracellular and intracellular signalling processes. While current, contrasting models for classic cadherin homophilic interaction involve varying numbers of specific repeats found in the extracellular domain, the structure of the domain itself clearly remains the main determinant of cell stability and binding specificity. Through intracellular interactions, cadherin enhances its adhesive properties binding the cytoskeleton via cytoplasmic associated factors α-catenin, β-catenin and $p120^{ctn}$. Recent structural studies on classic cadherins and these catenin molecules have provided new insight into the essential mechanisms underlying cadherin-mediated cell interaction and catenin-mediated cellular signalling. Remarkable structural diversity has been observed in β-catenin recognition of other cellular factors including APC, Tcf and ICAT, proteins that contribute to or compete with cadherin/catenin functioning. ***BioEssays*** **26:497–511, 2004.**

Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Ontario, Canada.

Funding agencies: J.G. is supported by a Premier's Research Excellence Award (to M.I.) while K.L.Y. is supported by a Canadian Institutes of Health Research (CIHR) Doctoral Research Award. This work is also in part funded by a grant (to M.I.) from the National Cancer Institute of Canada. M.I. is a CIHR Senior Investigator.

*Correspondence to: M. Ikura, Division of Molecular and Structural Biology, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, 610 University Avenue, Rm. 7-707, Toronto ON M5G 2M9, Canada. E-mail: mikura@uhnres.utoronto.ca

DOI 10.1002/bies.20033

Published online in Wiley InterScience (www.interscience.wiley.com).

Introduction

The dynamic cellular and morphological changes occurring during development of multicellular organisms depend upon precisely controlled mechanisms of cell adhesion. These adhesive mechanisms coordinate an extensive region of cell-to-cell and cell-to-cellular matrix contact, forming an ordered structure, such as that seen in epithelia (Fig. 1A). One of the most important and ubiquitous types of adhesive interactions in both vertebrate and invertebrate species is mediated by the family of calcium (Ca^{2+})-dependent cell adhesion glycoproteins, cadherin (~120 kDa).[1,2]

Numerous biological processes, including hemostasis, immunological response, inflammation, embryogenesis and development of neural tissue, rely on the ability of one adhesion molecule to selectively adhere to another through precise intermolecular interactions[3]. In the case of cadherins, highly regulated spatiotemporal sequences of cadherin expression[4] and function are vital to tissue morphogenesis,[5,6] providing the basis for histogenetic separation and segregation of embryonic cells[7] and later forming the epithelial layers of the skin and intestine. As regulators of morphogenesis, cadherins play a further role in synapse formation, membrane integration, polarization, cell sorting, migration, cytoskeleton interaction and post-translational modifications.[2] Mutations in cadherin-encoding genes, followed by downregulation of cadherin expression, often result in tissue disorder, cellular de-differentiation, increased invasiveness of tumor cells and ultimately malignancy.[8,9]

Among the numerous structurally and functionally diverse members of the cadherin superfamily, the classic (type I) cadherins are the best characterized and offer the richest insight into our understanding of the cadherin structure–function relationship. These transmembrane proteins contain three distinct domains: the extracellular (EC) domain, a hydrophobic membrane-spanning domain and an intracellular cytoplasmic domain (Fig. 1C). The classic cadherin extracellular portion consists of five tandem repeats of roughly 110 amino acids, which is used in classifying the different types of cadherin molecules. Type I cadherins, such as epithelial (E-) and neural (N-) cadherin, mediate both homotypic (one type of cadherin on one cell surface interacting with the same type of

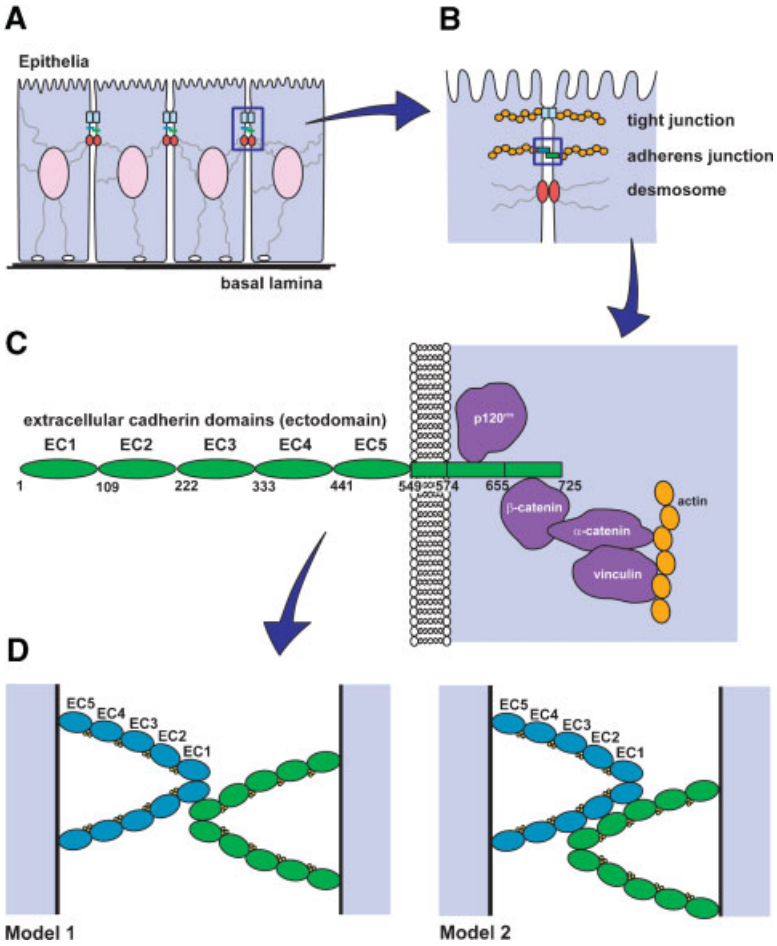

Figure 1. A: Adhesive structures in the epithelia. Cell-to-cell and cell-to-matrix adhesive contacts depicted in intestinal epithelial cells. Adapted from Tsukita et al.[98] **B:** Junctional complex. Three common types of junctions: tight, adherens and desmosomal. The adherens junction in an epithelial cell regulates the formation of the tight junction (light blue), and comprises two adjacent cadherin molecules (blue and green) in a homophilic interaction. The desmosome (red) attached to intermediate filaments (grey) is a highly organized structure composed of members from the cadherin, armadillo and plakin families.[31] **C:** Cadherin domain layout. A cadherin molecule (green) consists of five extracellular (EC) domains, a transmembrane domain and an intracellular domain, itself divided into a membrane proximal (residues 574–655) and catenin-binding (655–725) domain. In purple, interacting proteins $p120^{ctn}$ is bound to the membrane proximal region; β-catenin binds the catenin-binding domain, then binds α-catenin at its N terminus. α-catenin and vinculin form the direct link between the β-catenin–cadherin complex and the actin cytoskeleton (orange). Residue numbers shown are based on the sequence and structure of the C-cadherin ectodomain.[25] **D:** Homophilic interactions between *cis* dimers. Model 1 depicts the proposed single domain interface believed to be involved in homophilic cadherin binding;[25,47] the EC1 domain from one cadherin molecule (blue) interacts with the EC1 domain of a second cadherin molecule (green) protruding from the opposing cell surface. Model 2 depicts the proposed multi-interface interactions where homophilic cadherin adhesion involves three or more EC domains from two opposing molecules.[56] Ca^{2+} (yellow) binds to short, highly conserved amino acid sequences located between neighbouring extracellular repeats and is involved in domain rigidification for homophilic association.

cadherin on the surface of the opposing cell (Fig. 1B)) and heterotypic cell–cell interactions. While this extracellular domain is often sufficient to provide Ca^{2+}-dependent adhesion, interactions between the cadherin cytoplasmic tail and the cytoskeleton significantly increase the strength of cadherin-mediated adhesion.[10] The link to the cytoskeleton occurs via cytoplasmic domain interaction with a complex of β-catenin (92 kDa) (or γ-catenin/plakoglobin, 83 kDa), α-catenin (102 kDa) and vinculin (117 kDa)[11] (Fig. 1C). When unbound to β-catenin, the cytoplasmic domain of cadherin becomes unstructured.[12] In addition to its function in cadherin-based adhesion, β-catenin also plays a central role in the Wnt signalling pathway.[13,14] To this end, β-catenin is capable of binding numerous proteins, including members of the lym-

phoid enhancer-binding factor/T cell factor (Lef/Tcf)-family, adenomatous polyposis coli (APC), α-catenin, axin and the inhibitor of α-catenin and Tcf (ICAT). Elucidation of the three-dimensional structures of β-catenin alone and in association with some of these proteins reveals how β-catenin achieves its promiscuity[15] hereby acting as a modulator of cadherin-based adhesion.

This review provides a brief overview of the cadherin superfamily, outlines the current structural information available for classic cadherins and emphasizes the structure–function relationship of the cadherin–catenin complex. Furthermore, we highlight novel and important developments in the characterization of homophilic cadherin binding, the cadherin–Ca^{2+} interaction and β-catenin partner interaction. Some excellent reviews on cellular and physiological function as well as pathological roles of the various members of the cadherin superfamily have been published previously.[10,16–19]

The cadherin superfamily

The evolutionarily diverse cadherin protein superfamily consists of approximately 80 members[17] (Table 1). Aided by genome sequencing projects, an exact determination of the number of cadherin sequences in *Caenorhabditis elegans*[21] and *Drosophila melanogaster*[15] has recently been compiled[20]. Based on domain composition, genomic organization and overall structure, the superfamily is most often divided into six subgroups: (1) classical or type I cadherins, (2) the highly related classical type II cadherins, (3) desmosomal cadherins, (4) protocadherins, (5) Flamingo cadherins and (6) FAT-like cadherins. In addition to the cadherins that fall within defined subgroups, numerous atypical cadherin proteins occupy unique, isolated positions within the cadherin superfamily. To aid in cadherin classification, a web-based classification tool and database (http://calcium.uhnres.utoronto.ca/cadherin) has been made available.[21]

Classical or type I cadherins (E-, N-, P-, R-, H-, EP-cadherin) mediate adhesion at the adherens, cell–cell or cell–matrix adhesive junctions that are linked to microfilaments (Fig. 1B). A predomain (usually less than 80 amino acids) between the signal sequence and the start of the EC1 domain exists at the N terminus[22,23] and must be cleaved prior to adhesive function activation.[24] Following synthesis on the rough endoplasmic reticulum, the predomain is cleaved by convertases from the subtilisin/furin family revealing a prodomain sequence of about 130 amino acids, the position of which suggests that it functions by obstructing the adhesive interface.[25] As previously mentioned, type I classical cadherins are composed of five tandem extracellular cadherin domains (EC1–EC5), a single segment transmembrane domain and a distinct, highly conserved cytoplasmic tail that specifically binds catenins. Extracellular domains EC1 to EC4 are homologous cadherin repeats and include the well-known His-Ala-Val (HAV)-sequence (conserved within the binding surface of the first domain[26,27]), while EC5 is a less-related membrane-proximal domain.

The highly related atypical or type II cadherins (cadherin-VE, K, 7-12, 18, 19, 20), are expressed in loosely associated cells and are responsible for comparatively weaker intercellular adhesion.[28] Type II cadherins are more cell-type specific than type I cadherins, particularly during embryonic development.[9,29] Type II members also contain five extracellular domains and a highly conserved cytoplasmic tail, but the first of the EC domains lacks the HAV sequence believed to play a role in type I adhesion. The cytoplasmic tail of type II is also capable of interaction with β-catenin, although the strength of this interaction is significantly less than that observed within type I cadherins.[30]

Desmosomes are highly organized structures comprising members of the cadherin, armadillo and plakin protein families.[31] The desmosomal cadherins provide adhesion between cells in the desmosomal junctions (particularly those subjected to mechanical strain) and intermediate filaments.[3] There are two subfamilies of glycosylated desmosomal cadherins, the desmocollin (DSC) (90–100 kDa) and desmoglein (DSG, 130–165 kDa) proteins. Each group is further divided into three subtypes, which are expressed in a cell-type- and differentiation-specific manner. Both DSC and DSG consist of four cadherin domains in the extracellular portion of the molecule (EC1–EC4 domains), a membrane-proximal extracellular segment, a transmembrane domain and a cytoplasmic domain that is necessary for catenin binding. The catenin-binding C terminus of DSG is longer than that of classical cadherins[32] and contains a proline-rich region,[33] a unique terminal domain and a large repeating unit domain with unknown function.[34] DSC contains a cytoplasmic tail alternatively spliced into a longer "a" form and a shorter "b" form.[35] DSC and DSG in combination, but not individually, are strong mediators of Ca^{2+}-dependent homotypic cell-to-cell association, although each is capable of Ca^{2+}-independent heterotypic interaction.[35]

Protocadherins are a large subfamily of Ca^{2+}-dependent, cadherin-like adhesion proteins highly expressed in the synapses of the central nervous system. These proteins have up to seven extracellular domains, which lack the characteristic features of the classic cadherins. It has been suggested that the main function of protocadherins is not cell-to-cell adhesion activity, but the determination of specificity in cellular interactions and signal transduction.[36]

The most-novel type of cadherin, recently identified from *Drosophila* screens, is referred to as the Flamingo cadherin.[32] The extracellular portion of this protein consists of nine cadherin repeats. Each member also contains a cytoplasmic tail that lacks a catenin-binding site. In addition, the Flamingo cadherin has a unique seven transmembrane segment, unlike the single transmembrane segment observed in classical cadherins. Flamingos have been found to mediate homotypic

Table 1. The Cadherin Superfamily

Subfamily	# of EC domains	Cytoplasmic domains	Binds catenins (Yes/No)	Reference
Classical/type I Cadherins				Takeichi, M. Development. 1988. Apr;102(4):639–655. Review. Kemler, R. Semin Cell Biol. 1992. Jun;3(3):149–155. Review.
E-cadherin (Cadherin-1)	5	Type C	Yes	Nagafuchi, A., et al. Nature. 1987 Sep 24–30;329(6137):341–343. Mansouri, A., et al. Differentiation. 1988 Jun;38(1):67–71.
N-cadherin (Cadherin-2)	5	Type C	Yes	Hatta, K., et al. J Cell Biol. 1988 Mar;106(3):873–881.
P-cadherin (Cadherin-3)	5	Type C	Yes	Nose, A., Takeichi, M. J Cell Biol. 1986. Dec;103(6 Pt 2):2649–2658.
R-cadherin (Cadherin-4)	5	Type C	Yes	Inuzuka, H., Miyatani, S., Takeichi, M. Neuron. 1991 Jul;7(1):69–79.
H-Cadherin (Cadherin-13)	5	Type C	Yes	Lee, S.W., et al. Nat Med. 1996. 2:776–782.
Cadherin-15	5	Type C	Yes	Shimoyama, Y., et al. J Biol Chem. 1998. Apr 17;273(16):10011–10018.
EP-cadherin	5	Type C	Yes	Ginsberg, D., DeSimone, D., Geiger, B. Development. 1991 Feb;111(2):315–325.
C-cadherin	5	Type C	Yes	Brieher, W.M., and B.M. Gumbiner. J. Cell Biol. 1994. 126:519–527.
DE-cadherin (*Drosophila*)	6[1]	Type C	Yes	Oda, H., et al. Dev Biol. 1994. 165, pp. 716–726.
Type II Cadherins	No HAV			Yagi, T, Takeichi, M. Genes Dev. 2000. May 15;14(10):1169–1180. Review. Angst, B.D., Marcozzi, C., Magee, A.I. J Cell Sci. 2001. Feb;114 (Pt 4):629–641. Review.
VE-cadherin (Cadherin-5)	5	Type C	Yes	Tanihara, H., et al. J Cell Sci. 1994. 107 (Pt 6): p. 1697–1704.
K-cadherin (Cadherin-6)	5	Type C	Yes	Xiang, Y.Y., et al. Cancer Res. 1994. Jun 1;54(11):3034–3041.
Cadherin-7	5	Type C	Yes	Shimoyama, Y., et al. Biochem J. 2000. Jul 1;349(Pt 1):159–167. Kools, P., Van Imschoot, G., van Roy, F. Genomics. 2000. Sep 15;68(3):283–295.
Cadherin-8	5	Type C	Yes	Shimoyama, Y., et al., Biochem J. 2000. Jul 1;349(Pt 1):159–167.
Cadherin-9	5	Type C	Yes	Shimoyama, Y., et al., Biochem J. 2000. Jul 1;349(Pt 1):159–167.
Cadherin-10	5	Type C	Yes	Fushimi, D., Dev Dyn. 1997. Jul;209(3):269–285.
OB-cadherin (Cadherin-11)	5	Type C	Yes	Okazaki, M., et al., J Biol Chem. 1994. Apr 22;269(16):12092–12098.
Cadherin-12	5	Type C	Yes	Tanihara H., et al., Cell Adhes Commun. 1994. Apr;2(1):15–26.
Cadherin-18	5	Type C	Yes	Nollet, F., Kools, P., van Roy, F. J Mol Biol. 2000. Jun 9;299(3):551–572.
Cadherin-19	5	Type C	Yes	Kools, P., Van Imschoot, G., van Roy, F. Genomics. 2000. Sep 15;68(3):283–295.
Cadherin-20	5	Type C	Yes	Kools, P., Van Imschoot, G., van Roy, F. Genomics. 2000. Sep 15;68(3):283–295.
Cadherin-24	5	Type C	Yes	Katafiasz, B.J., et al. J Biol Chem. 2003 Jul 25;278(30):27513–27519.
Desmosomal Cadherins				Buxton, R.S., Magee, A.I. Semin Cell Biol. 1992. Jun;3(3):157–167. Wheeler, G.N., et al., Biochem Soc Trans. 1991. Nov;19(4):1060–1064. Review.
Desmocollin-1-3	5	Type D	Yes	Collins, J.E., et al. J Cell Biol. 1991 Apr;113(2):381–391.
Desmoglein-1-3	5	Type D	Yes	Koch, P.J., et al. Eur J Cell Biol. 1990. Oct;53(1):1–12.
Protocadherins				Suzuki, S.T. Exp Cell Res. 2000. 261(1): p. 13–18. Sano, K., et al. Embo J. 1993. 12(6): p. 2249–2256. Suzuki, S.T. J Cell Sci. 1996. Nov;109 (Pt 11):2609–2611.
α-protocadherin	6	Type P	No	Wu Q, Maniatis T. Cell. 1999 Jun 11;97(6):779–790.
β-protocadherin	6	Type P	No	Wu Q, Maniatis T. Cell. 1999 Jun 11;97(6):779–790.
γ-protocadherin	7	Type P	No	Wu Q, Maniatis T. Cell. 1999 Jun 11;97(6):779–790.
*Pcdh*1 (cadherin-like 1)	7	Type P	No	Obata, S., et al. J Cell Sci. 1995 Dec;108 (Pt 12):3765–3773.
*Pcdh*2	?	Type P	No	Obata, S., et al. J Cell Sci. 1995 Dec;108 (Pt 12):3765–3773.
*Pcdh*3	6	Type P	No	Sago, H., et al. Genomics. 1995. Oct 10;29(3):631–640.
*Pcdh*7(*BH protocadherin*)	7	Type P	No	Yoshida, K., et al. Genomics. 1998. May 1;49(3):458–461.
*Pcdh*8	6	Type P1	No	Strehl, S., et al. Genomics. 1998. Oct 1;53(1):81–89.
*Pcdh*9	6	Type P/P2	No	Strehl, S., et al. Genomics. 1998. Oct 1;53(1):81–89.
*Pcdh*10	6	Type P1	No	Wolverton T, Lalande M. Genomics. 2001 Aug;76(1–3):66–72.
*Pchd*11	7	Type P1	No	Yoshida K, Sugano S. Genomics. 1999 Dec 15;62(3):540–543.
*Pcdh*18	6	Type P1	No	Wolverton T, Lalande M. Genomics. 2001 Aug;76(1–3):66–72.
*Pcdh*19	6	Type P1	No	Wolverton T, Lalande M. Genomics. 2001 Aug;76(1–3):66–72.
Papc	6	Type P2	No	Yamamoto, A., et al. Development. 1998. Sep;125(17):3389–3397.

(*Continued*)

Table 1. (*Continued*)

Subfamily	# of EC domains	Cytoplasmic domains	Binds catenins (Yes/No)	Reference
NF-protocadherin (*Xenopus*)	?	Type P3	No	Bradley, R.S., Espeseth, A., and Kintner, C. Curr. Biol. 1998. 8, 325–334.
CNRs	6	Type P4	No	
Seven-pass transmembrane cadherins (Flamingo (fmi)/ Starry Night)				Takeichi, M., et al. Philos Trans R Soc Lond B Biol Sci. 2000. Jul 29;355 (1399): 885–890. Review. Bray S. Curr Biol. 2000. Feb 24;10(4):R155–R158. Review.
Fmi (Xenopus, *Drosophila*, *human*)	9	Type S	No	Usui, T., et al. Cell. 1999. 98(5): p. 585–595.
Celsr1 (mouse Fmi)	9	Type S	No	Formstone, C.J., Little P.F. Mech Dev. 2001. Nov;109(1):91–94. Hadjantonakis, A.K., et al. Genomics. 1997. Oct 1;45(1):97–104.
Fat-like cadherins				Nollet, F., Kools, P., van Roy, F. J Mol Biol. 2000. Jun 9;299(3):551–572. Review.
FAT (*Drosophila*)	34[2]	Type F	Yes	Mahoney, P.A., et al. Cell. 1991. Nov 29;67(5):853–868.
FAT (Human—hFAT1 and hFAT2)	34	Type FH	Yes	Dunne, J, et al. Genomics. 1995. Nov 20;30(2):207–223.
Dachsous (*Drosophila*)	27	Type FD	Yes	Clark, H.F., et al. Genes Dev. 1995. Jun 15;9(12):1530–1542.
Unique Cadherins				
T-cadherin	5	None	No	Ranscht, B. and M.T. Dours-Zimmermann. Neuron. 1991. 7(3): p. 391–402.

[1]5 domains are present after maturation.
[2]Lysine-alanine-leucine instead of the HAV sequence.
Type C = a highly conserved cytoplasmic domain that interacts with beta-catenin and p120 catenin.
Type P = constant cytoplasmic domain which bears no similarity to classical cadherins or to other members of the protocadherin family (or other isoforms of the same protocadherin) and represents a novel sequence.
Type P1 = See Type P. Contains the unique 17-amino acid motif (CM-2) first found in Pcdh8.
Type P2 = See Type P. Contains a highly conserved stretch of 26 amino acids in the middle of the cytoplasmic domain, which is rich in Ser and Asp
Type P3 = binds the cellular protein TAF1, previously identified as a histone-associated protein.
Type P4 = distinct cytoplasmic domain that interacts specifically with the tyrosine kinase Fyn.
Type D = a novel 282-residue extension cytoplasmic extension, which contains repeats of approximately 29 amino acid residues predicted to have an antiparallel beta-sheet structure, followed by a glycine-rich sequence. The cytoplasmic region spans the desmosomal plaque at least once. A 37 residues stretch is required to plakoglobin, as well as of desmoplakin, and to act as an intermediate filament anchorage.
Type S = an anonymous intracellular C terminus. Little is known about the flamingo cytoplasmic domain, except for Celsr1–3, the flamingos found in mice. Celsr1 and 2 have a cytoplasmic tail of about 300 amino acids, whereas that of Celsr3 is 590 residues long. Apart from a proline-rich stretch in Celsr3, the cytoplasmic sequences do not contain any conserved motif.
Type F = novel cytoplasmic domain containing one domain with distant homology to the cadherin-binding region of classical cadherins.
Type FH = 386 residue acidic and proline-rich sequence contains two domains with distant homology to the cadherin catenin-binding region.
Type FD = sequence similarity to the cytoplasmic beta-catenin-binding domain of classic vertebrate cadherins.

adhesion and are involved in the establishment of cell polarity.[37,38]

First identified as a tumour-suppressor protein in *Drosophila*, FAT contains 34 extracellular repeats.[32,39] The cytoplasmic domain of FAT contains sequences homologous to the β-catenin-binding region found in classic cadherins as well as a putative PDZ-domain binding sequence (TEV, conforming to the X-Thr/Ser-X-Val-COOH motif),[39,40] which mediates protein–protein interactions at the plasma membrane.[41] The TEV sequence is not conserved in *Drosphilia*, but is conserved in the mammalian FAT sequences of humans and rats.[40] Given the large size of the extracellular portion, it has been suggested that FAT cadherin plays a role outside of adhesion, and is possibly involved in cell repulsion, acting as a sensor of cell-to-cell proximity and a brake on cell proliferation.[42]

Truncated (T-)cadherin (or cadherin-13), a unique member within the cadherin superfamily, shares its ectodomain organization with classical cadherins, but lacks both the transmembrane and cytoplasmic regions. T-cadherin is instead modified with a glycosyl-phosphatidylinositol (GPI) moiety that anchors it to the plasma membrane.[32] T-cadherin, originally cloned from chicken embryo brain,[43] mediates Ca^{2+}-dependent adhesion but is not limited to cell-to-cell contacts as seen in type I molecules. It has recently been suggested that T-cadherin is also involved in cell signalling.[44,45]

Basic molecular structure of cadherin

Over the past decade, countless contributions have been made to increase our present understanding of the exact molecular architecture of cadherins. The structures of the EC1

domain of mouse E-cadherin and N-cadherin were determined by NMR spectroscopy[46] and X-ray crystallography[47] in 1995 (Fig. 2A,B). These structures reveal a folding topology similar to an immunoglobulin variable-like domain[46,47] not originally predicted from the sequence. Both N- and E-cadherin EC1 domains show an overall structure of a seven-stranded β-sheet or β sandwich (A′, B, C, D, E, F, and G)[46] with the amino and carboxyl termini located at opposite ends of the molecule and Ca^{2+} ions bound to the loops adjoining individual domains.[24] The segment connecting strands B and C adopts a helical structure of successive β-turns and β-like hydrogen bonds. This unique quasi-β-helix structure is characteristic of the EC1 domain of classical and desmosomal cadherins.[48] Following the determination of such single domain structures, crystal structures of E-cadherin and N-cadherin containing both the first and second domains were reported.[49–51] Within these structures, Ca^{2+} binding to the extracellular portion of the polypeptide chain was determined to be a prerequisite for cadherin-mediated cell adhesion[9] (Fig. 3). It was proposed, based on the crystal structure of E-cadherin EC1/EC2, that Ca^{2+} binding acts to rigidify the two-domain structure, thereby fixing domain orientation.[49] A ten-residue linker region between EC1 and EC2 binds three Ca^{2+} ions, with six Ca^{2+} ions found in the dimeric structure[49] (Fig. 2C). Electron microscopic studies on a recombinant E-cadherin EC1–5 construct[50] also suggest that calcium concentration plays a role in cadherin dimer formation. Recently, the structure of the entire extracellular region (the ectodomain) of C-cadherin was elucidated[25] (Fig. 2D). The ectodomain is elongated but substantially curved such that the long axis of EC1 is approximately perpendicular to the long axis of EC5. In this structure, the quasi-β-helix of EC1 coordinates Ca^{2+} in the EC2–EC3 linker region via conserved acidic residues. Each EC domain adopts a Greek key fold, with the interdomain boundaries rigidified by three ligated Ca^{2+} ions.[25]

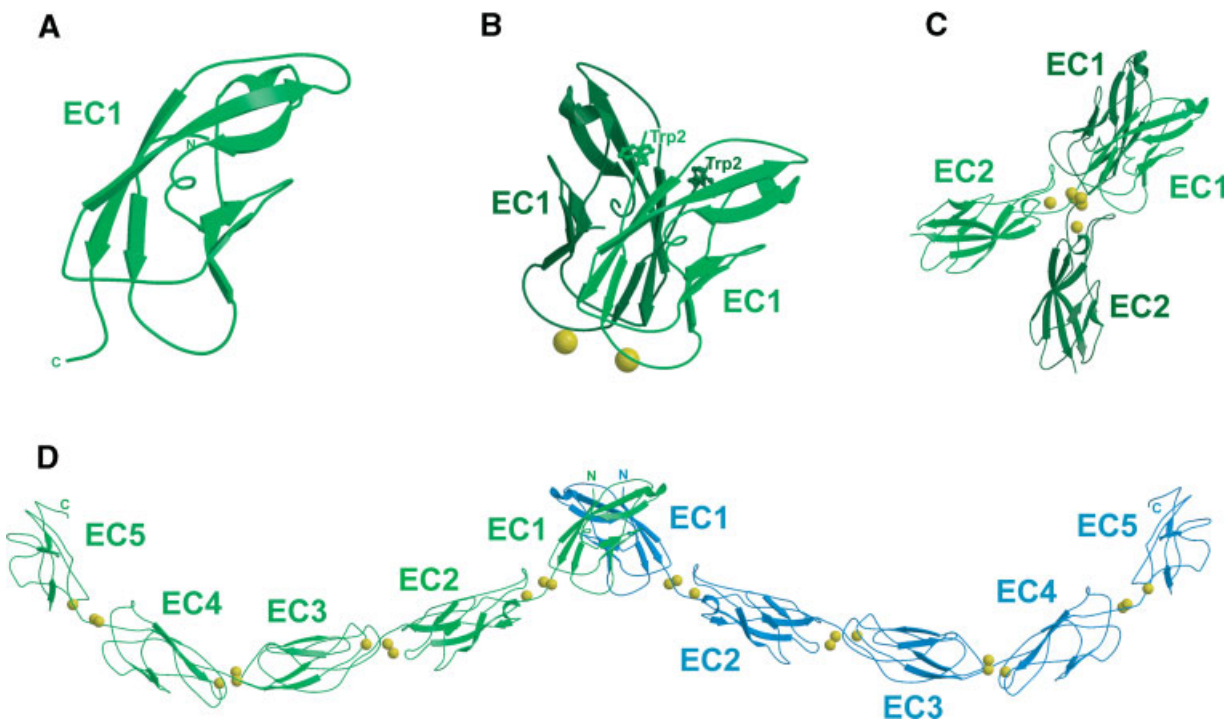

Figure 2. A: E-cadherin EC1 domain. The EC1 domain shows an overall structure of a seven strand β-sheet with the amino and carboxyl termini located at opposite ends of the molecule. This unique quasi-β-helix structure is characteristic of the EC1 domain (PDB code 1SUH).[46,99] **B:** N-cadherin EC1 domain interface. One EC1 monomer, depicted in green, forms a *cis* dimer with a second EC1 monomer depicted in dark green; both protrude from the same cell surface (1NCG).[47] **C:** Dimeric structure of E-cadherin EC1–EC2 dual domain. The EC1 and EC2 domains from two cadherin monomers (one in light green and the other in dark green) from the same cell surface interact to form a dimer (1EDH). A cluster of three Ca^{2+} ions is bound in the linker region connecting the N- and C-terminal domains of each molecule.[49] **D:** Proposed dimeric structure C-cadherin EC1–EC5 domains. Two C-cadherin ectodomains from adjacent cell surfaces interact to form a strand dimer (accession code 1L3W).[25]

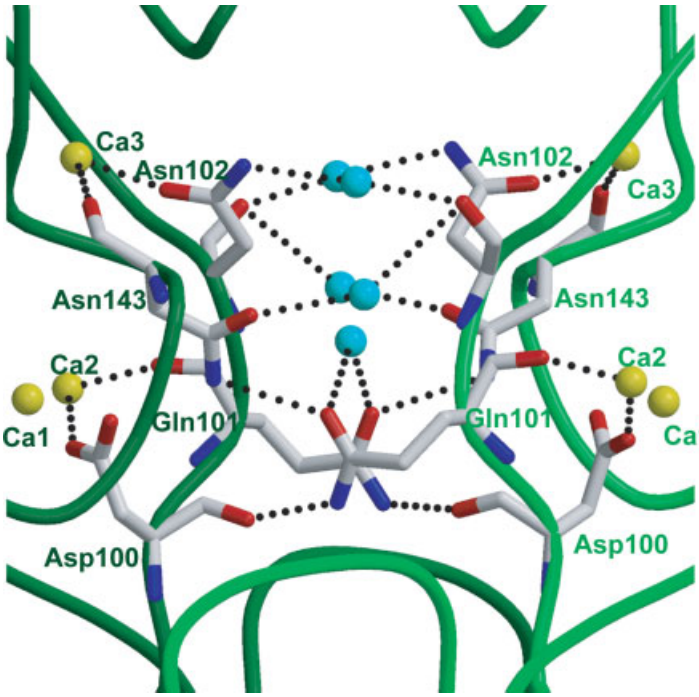

Figure 3. Calcium coordination sites in E-cadherin EC1-EC2 dual domain. The Ca^{2+}-binding region is shown between two cadherin monomers on the same cell surface (light and dark green), Ca^{2+} ions Ca1, Ca2, and Ca3 (yellow) and coordinating water molecules (blue). Selected hydrogen bonds and side chain interactions involved in dimer formation are depicted. Carbonyl oxygen atoms are shown for each residue whose side chain is emphasized. Adapted from Figure 2c in Nagar B, et al.[49]

Extracellular domain interaction of cadherins

Cis *and* trans *interactions*

Much focus has been placed on identifying the molecular interfaces that mediate the association of classic cadherins. Following mutational and adhesion-inhibition studies, homotypic adhesion involving a single domain interface, EC1, was proposed to describe the cadherin–cadherin interaction. This early research suggested that Trp2 and the conserved HAV sequence were essential components of the EC1 adhesion recognition site.[27] X-ray crystallographic studies of the same N-cadherin EC1 fragment[47] showed the N-terminal Trp2 inserting into a pocket formed by hydrophobic residues surrounding the HAV sequence and, as a result, a two-step mechanism for cadherin association was proposed.[47] First, a strand dimer was believed to form via intermolecular interactions between the N-terminal residues, which involve Trp2-mediated *cis* interaction[47] (Fig. 2B). It was then proposed that this *cis*-dimerized pair undergoes a *trans* interaction forming the 'adhesion dimer' with a complementary antiparallel or adjacent cadherin molecule (Fig. 1D). Alternating *cis* and *trans* interactions thus produce a zipper-like superstructure.

However, in later two-domain structures of E- and N-cadherin,[49,51] these contacts were not observed. Instead, the closest contact was seen in the Ca^{2+}-binding region of the proposed intertwisted X-shaped dimer. The distance between the two molecules was decidedly too large for the strand exchange observed in the N-cadherin EC1 structure. In addition, Pertz et al. reported a crystal structure of an E-cadherin ectodomain fused to the coiled-coil assembly domain of cartilage oligomeric matrix protein (ECADCOMP).[50] In this structure, the side chains of residues involved in Ca^{2+} coordination are the same as those found in the EC1/EC2 E-cadherin structure.[49] Trp2, however, inserts into a hydrophobic pocket formed by residues of its own polypeptide chain, and not into the pocket of the adjacent molecule as observed in the crystal structure of the single domain fragment of N-cadherin.[47] The insertion was believed to induce a conformational change at a distinct adhesion site. Based on these observations, the authors suggested that the *cis* dimers from two different ECADCOMP molecules coordinate to form a single *trans* interaction.[50]

The most-recently determined crystal structure, the entire C-cadherin ectodomain[25] (Fig. 2D), again displays the two-fold symmetric exchange of the N-terminal β-strands (Trp2) between the EC1 domains of neighbouring molecules but, surprisingly, the strand dimer in this structure is placed in the *trans* orientation as opposed to the *cis* orientation observed in the first structures of N-cadherin EC1 domain.[47] The interface also involves more extensive interactions, including: (i) a salt bridge between the side chain of Glu89, conserved in classical cadherins, and the N terminus,

(ii) hydrogen bonding between Trp2 and the backbone carbonyl of Glu90, and (iii) hydrogen bonding between residues Asp1 to Val3 of the A strand and residues Lys27 to Asn25 of the partner B strand.[25]

Multiple-domain integration

Several new models of homophilic cadherin binding, which suggest multiple adhesive interactions, add to the controversy surrounding the cadherin contact regions. Biophysical studies measuring direct molecular force between cadherin ectodomains suggest that more than the EC1 domain is required for homophilic binding.[52,53] Sivasankar et al. demonstrated that the strongest interaction was detected when the antiparallel proteins fully overlapped, leading to extensive overlay of multiple EC domains. The authors speculate that the orientation would enable a pair of ectodomains from opposing cells, by adhering and sliding successively along the molecular axis, to remain attached while being pulled apart. In this way, abrupt failure of the junction can be delayed.[52,53] More recently, by measuring both the absolute distance at which opposed cadherin fragments bind and the changes in the interaction potentials resulting from deletions of individual domains, it was shown that at least two domains participate in homophilic cadherin binding.[54] In this case, a novel, modular binding mechanism was suggested in which opposed cadherin ectodomains adhere in three antiparallel alignments (EC1–EC3, EC1–EC4, EC1–EC5). Based on a range of protein binding (both homo- and heterophilic) relative to protein dimensions, the strongest bond was seen between interacting EC3 domains.[54] The observed outermost bond corresponds to the distance of direct EC1–EC1 contact, in agreement with the proposed EC1 involvement in the *trans* interaction.[25,47,55] From these studies, it was concluded that two cadherin domains, EC1 and EC3, participate in binding, although this remains a matter of debate.[54]

Bead aggregation and adhesion assays of cadherin molecules lacking successive or individual EC domains suggest that three or more extracellular repeats are required to achieve full adhesive capacity (Fig. 1D).[56] Homophilic adhesion mediated by an EC1–EC2 construct exhibited very low adhesive activity when compared to the full ectodomain (EC1–EC5), demonstrating that EC1 alone is not sufficient for effective homophilic binding.[56] In accordance with these results, EC1–EC3 and EC1–EC4 constructs were seen to exhibit higher activity than the EC1–EC2 construct, but still less than EC1–EC5. Notably, EC3 does not appear to be required for homophilic binding with constructs lacking EC3 exhibiting high adhesive activity (EC1–EC2–EC4 and EC1–EC2–EC4–EC5).[56] Together, these findings suggest that the homophilic interactions formed between cadherins involve extensive overlap between the extracellular domains and may arise from multiple interactions or different combinations of interactions between EC domains.

Another study consistent with a multiple domain interface showed that the deletion of the outer N-terminal domain of epithelial cadherin did not abolish adhesion to full-length cadherin, and inverting the HAV motif did not impair adhesion in E-cadherin-mediated cell aggregation.[26] Most recently, electron tomography of plastic sections of neonatal mouse skin, show cadherin molecules forming discrete groups and interacting through their tips.[57] The resulting three-dimensional maps closely corresponded with the structure of the C-cadherin dimer previously proposed in which Trp2 is inserted into the hydrophobic pocket of the EC1 domain of the partner molecule. These studies further supported that Trp2 exchange is responsible for both *cis* and *trans* interactions; however, the initial Trp2 insertion would be followed by a compaction step in which networks of molecules combine to form large adhesive knots producing full adhesion. The physical proximity of molecules within these groups suggests that complementary faces of various EC domains interact to produce a knot of entwined molecules at the midline, with full adhesion resulting from the combined effect of a large number of diverse weak interactions.[57]

Viewed together these results suggest that many, if not all, EC domains contribute to the formation of the cadherin homophilic interaction, contrasting early studies where specific one-to-one interaction between EC1 domains was required. It should be noted that there are many possible models for homophilic cadherin interaction that fall within the scope of single or multiple domain adhesive models, including a possible EC1–EC4 or EC1–EC5 interaction not discussed above. It is also unclear whether the interactions involving the EC1 domain or multiple EC domains occur under physiological conditions or are possible only in some crystalline states.

Cadherin interaction with diverse partners

In addition to cadherin homophilic binding, it has been reported that cadherin is also capable of heterophilic interactions with numerous extracellular and intracellular proteins. T-cells expressing integrins $\alpha E\beta 7/\alpha M290\beta 7$ specifically interact with E-cadherin in the lymphocyte adhesion system.[58–60] N-cadherin is capable of binding the FGF receptor-1 (FGFR1), a molecule implicated in the differentiation of endothelial cells and neuronal cells.[61] Most recently, E-cadherin was found to bind presenilin-1,[62] CREB binding protein (CBP),[63] and the listerial protein internalin (InlA).[64] The structure of the functional domain of InlA (residues 36–496) in complex with EC1 of human E-cadherin (hEC1)[65] (Fig. 4) revealed a unique mode of molecular recognition. hEC1 occupies InlA's central cavity created by the 16-strand parallel β-sheet of its curved leucine-rich repeat domain. In this complex, InlA primarily binds the first two β-strands of hEC1 and the interconnecting loop, suggesting that the hEC1 surface is the most exposed region of the molecule, and supporting its identification as the *trans* interaction surface.[25] It is interesting to speculate that these heterophilic cadherin interactions

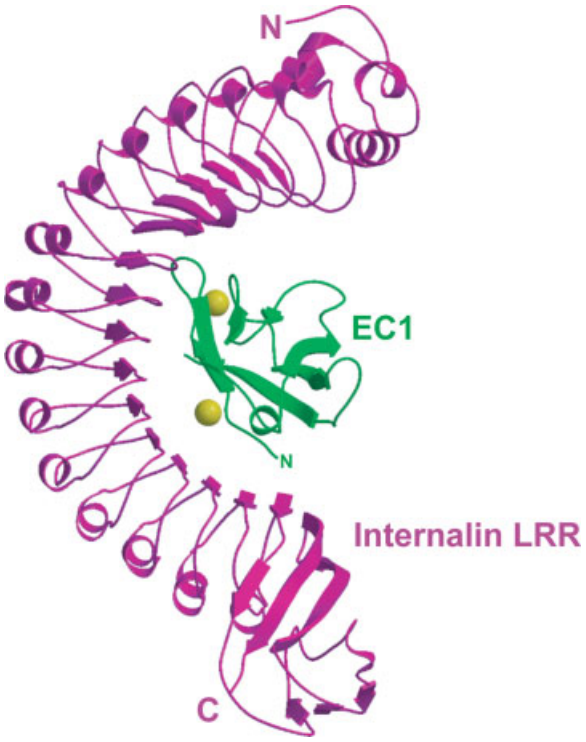

Figure 4. InlA (residues 36–496) in complex with hEC1. hEC1 (green) occupies the central cavity created by InlA's (magenta) curved leucine-rich repeat (LRR). The LLR consists of fifteen and a half 22 residue repeats. The InlA cavity of a 16-stranded parallel β-sheet covers more than 180° of the circumference of hEC1.

can occur in other cadherin superfamily members, expanding the role of cadherin in complex adhesion interactions.[28]

Cytoplasmic domain interactions of cadherin

The cytoplasmic domain plays a crucial role in cell adhesion through extracellular lateral clustering and cytoskeleton interaction.[66] The highly conserved, serine-rich, cytoplasmic region of approximately 70 amino acids is divided into two domains, a conserved membrane proximal domain and a catenin-binding sequence. Key to adhesive activity is the interaction between the catenin-binding sequence and submembrane plaque proteins β-catenin or plakoglobin (γ-catenin), which form the link to the actin cytoskeleton via α-catenin (Fig. 1C).

β-catenin's primary structure includes an N-terminal region of 149 residues, a central domain of 515 residues, which forms 12 armadillo repeats, and a 108-residue C-terminal segment. Each armadillo repeat consists of three helices, which stack to form a positively charged right-handed superhelix of helices. β-catenin recognizes a number of its binding partners as elongated peptides, through 'quasi-independent' subsites.[15] α-catenin binds to a short region close to the N terminus of β-catenin (residues 118–149[67,68]), forming a stable bond between the complex and the actin cytoskeleton. While the importance of β-catenin is discussed in detail below, the precise cellular role of γ-catenin, which shares very high sequence homology with β-catenin, has not been clearly defined. γ-catenin has, however, been identified in the zonula adherens junctions and a role in the desmosomal plaques has been suggested.[69,70]

In addition to α-, β-, and γ-catenin, a fourth catenin-like protein capable of binding cadherin, $p120^{ctn}$, has emerged as a key regulator of cadherin function.[71] $p120^{ctn}$ was originally identified as a substrate for receptor tyrosine kinases, including src,[72] and belongs to a discrete subfamily of proteins containing the armadillo repeat.[73] Like the other catenin molecules, $p120^{ctn}$ binds directly to the cytoplasmic domain of cadherin,[72] although binding occurs at the conserved membrane-proximal domain (Fig. 1C). Indirect evidence suggests a cooperative interaction between $p120^{ctn}$ and E-cadherin where $p120^{ctn}$, through various signalling events, affects cadherin adhesiveness both negatively and positively.[74] The $p120^{ctn}$–E-cadherin interaction may itself be sufficient to stabilize the E-cadherin complex or, alternatively, through inhibition of RhoA and activation of Rac[75] and Cdc42 (actin cytoskeleton modulators), $p120^{ctn}$ may promote the local assembly and organization of the actin cytoskeleton, supporting cadherin-bound $p120^{ctn}$ involvement in actin-driven cadherin clustering.[74] This positive adhesion regulation is suggested to be the principal function of $p120^{ctn}$.[74] The $p120^{ctn}$ NH_2-terminal regulatory region containing the phosphorylation domain is likely to act as a "dimmer switch" providing a mechanism for signalling pathways to regulate cadherin functions through post-translational modification of $p120^{ctn}$.[74]

The E-cadherin/β-catenin complex: phosphorylation effects

It has been shown that phosphorylation of the E-cadherin cytoplasmic domain enhances β-catenin-binding activity thus strengthening cell adhesiveness.[76] Structures of both unphosphorylated and phosphorylated cytoplasmic domains of E-cadherin (residues 577–728 of the mature E-cadherin sequence) in complex with the armadillo region of β-catenin (residues 134–671) have been determined[12] (Fig. 5). The crystal structure of unphosphorylated cadherin shows an extensive interaction surface (~6100 $Å^2$ with 2900 $Å^2$ contributed by β-catenin and 3200 $Å^2$ by E-cadherin) consisting of multiple, quasi-independent binding regions that span the entire length of the β-catenin repeats. The C-terminal residues of the cadherin cytoplasmic domain are crucial for β-catenin–cadherin complex formation, in particular residues 667–684, which form a highly conserved classical cadherin sequence. This interaction occurs via a salt bridge between

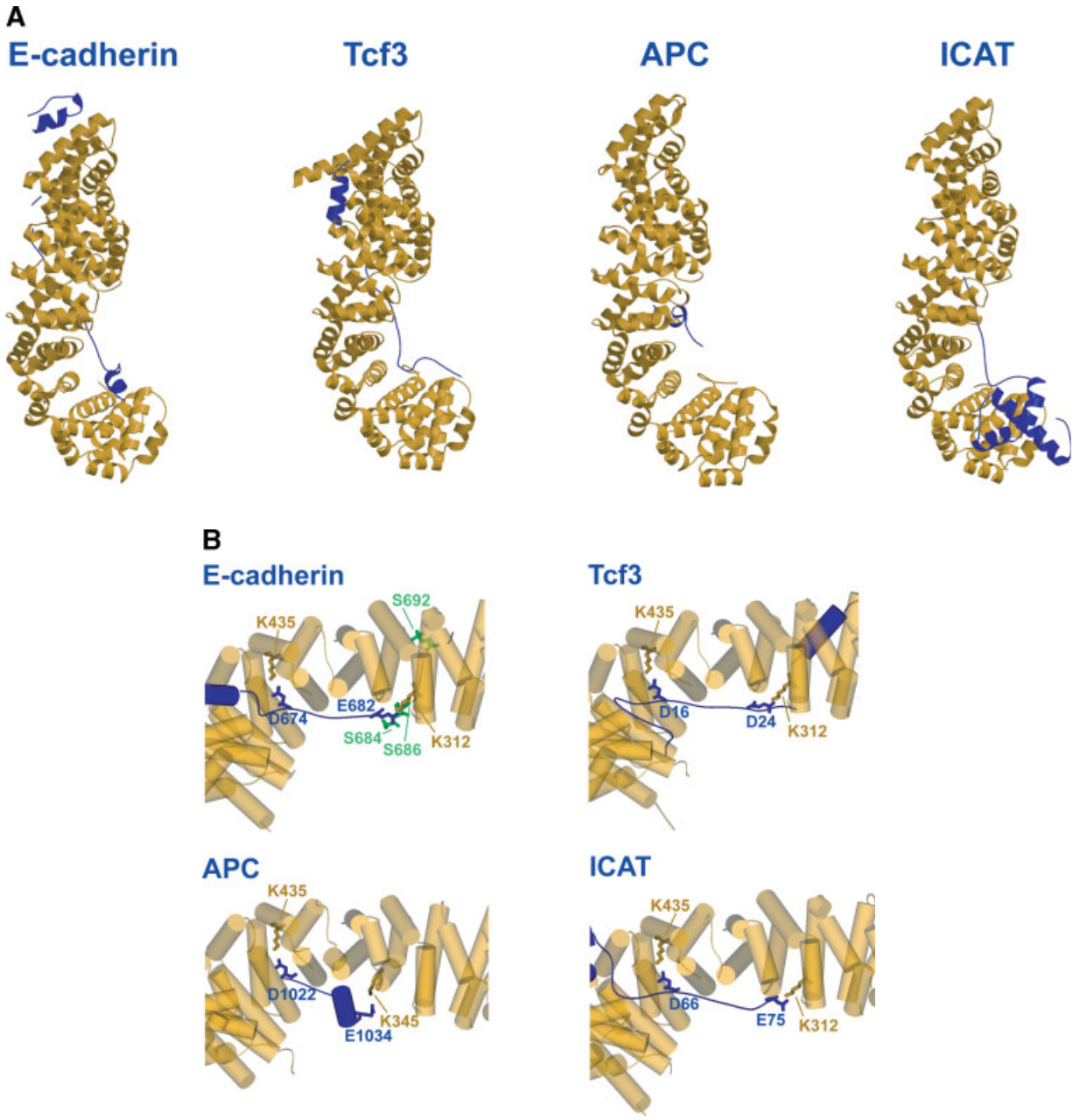

Figure 5. A: β-catenin and its binding partners. β-catenin (orange) is shown bound to several binding partners (blue). From left to right: E-cadherin (PDB code 1I7W), Tcf3 (1G3J), APC (IJPP) and ICAT (1LUJ). The β-catenin N-terminal region of 149 residues is located at the top of the diagram. The β-catenin central domain (515 residues) contains the 12 armadillo repeats, each consisting of three helices (residues 134–688), stacking to form a positively charged right-handed superhelix of helices. β-catenin's C terminus is located at the bottom of the diagram and is 108 residues in length. Note: breaks in the blue ribbon of E-cadherin are due to missing residues in the structure. **B:** Essential amino acid residues in β-catenin interactions. Key β-catenin residues are highlighted in orange. Essential glutamic acid and phosphorylated serine residues of the β-catenin-binding partners are shown in blue and green, respectively. Top left, essential to the β-catenin–E-cadherin interaction is salt bridge formation between cadherin residues Asp674 and Glu682 and two β-catenin charged buttons, Lys435 and Lys312, respectively. Top right, the same charged buttons of β-catenin interact with Glu24 and Asp16 of the XTcf3-CBD. Bottom left, only one common β-catenin charged button, Lys435, binds APC at Asp1022. In this complex, another button, β-catenin Lys345, forms a salt bridge with Glu1034. Bottom right, β-catenin–ICAT binding occurs along β-catenin armadillo repeats 5–12. The three-helix bundle of ICAT binds armadillo repeats 10–12 of β-catenin, anchoring ICAT to repeat 12. Second, a C-terminal tail binds armadillo repeats 5–9. In the ICAT tail domain, Asp66 forms a salt bridge with β-catenin Lys435 and Glu75 with β-catenin Lys312.

Asp674 and Glu682 in cadherin and Lys435 and Lys312, the so-called ''charged buttons'', in β-catenin. E-cadherin and α-catenin sit on roughly opposite surfaces of β-catenin, with both the N terminus of β-catenin and α-catenin pointing away from the plasma membrane.[77] The structure and interacting surfaces of phosphorylated cadherin complexed with β-catenin are essentially the same as those of the unphosphorylated form, except that a segment containing the phosphorylation sites (Ser684, Ser686, and Ser692) in the unphosphorylated form is not visible (Fig. 5B). This presumably flexible region (designated region IV[12]) becomes visible in the phosphorylated form, suggesting that phosphorylation of these serines stabilizes the structure of this region, a prerequisite to tighter binding with β-catenin. In other words, phosphorylation of these residues promotes induced folding probably upon complexation with β-catenin by formation of favourable side-chain interactions.

Region IV contains several serine residues in consensus positions for casein kinase II (CKII) and glycogen synthase kinase 3β (GSK-3β) phosphorylation. Serine-to-alanine mutation of CKII sites Ser684, Ser697 and Ser699 that abolish phosphorylation also decrease the amount of β-catenin bound to E-cadherin in vitro, suggesting that phosphorylation of these residues influences the strength of adhesion.[78] Interestingly, the CKII phosphorylation site in the crystal structure (Ser684) is visible in the unphosphorylated structure, but is not involved in β-catenin interaction.[12] Interaction of the two proteins can also be reduced by phosphorylation of β-catenin Tyr654.[79] Such regulation of cadherin–catenin complex formation provides a link between cadherin's role in signal transduction and intercellular adhesion.[80]

β-catenin's interaction with cellular partners

Over the past few years, a fascinating picture has emerged of cadherin and β-catenin involvement in the Wnt growth factor signalling pathway.[81] This pathway, in which β-catenin acts as a transcriptional coactivator, plays a key role in the control of cell differentiation and development, as well as malignancy.[13,14] In the absence of Wnt, and following phosphorylation by GSK-3β, free β-catenin (not in adherens junctions) is targeted for degradation by a large, multiprotein complex containing β-catenin, GSK3β, APC and scaffold protein axin.[82,83] When present, Wnt inhibits GSK3β-mediated phosphorylation of β-catenin and facilitates transport of β-catenin into the nucleus where it activates transcription through complex formation with transcription factors of the Lef/Tcf family.[84]

The β-catenin–Tcf complex

The majority of β-catenin is localized to the cell membrane where it mediates cadherin-based cell adhesion.[85] There is, however, a small pool of free β-catenin, found in both the nucleus and the cytoplasm that plays a role in the Wnt signalling pathway. Stimulation of the Wnt pathway results in the accumulation of the β-catenin–Tcf complex, which in turn is required for transcriptional activation of Wnt target genes modulating embryonic development and tumorigenesis. The crystal structure of β-catenin bound to the β-catenin binding domain of Tcf3 (XTcf3-CBD, residues 1–61 of *Xenopus* Tcf3) reveals an elongated structure with three binding modules: an N-terminal β hairpin (residues 7–15), an extended region (residues 16–29) and an α helix (residues 40–52) (Fig. 5).[78] These modules run antiparallel to β-catenin along the positively charged groove formed by the armadillo repeats. The hairpin region, which fits in the groove formed by armadillo repeats 9 and 10, is largely dispensable in β-catenin binding.[86] However, three ''hot spots'' at the β-catenin–Tcf3 interface are necessary for binding: one in the helical region and two charged buttons in the extended region. Thus, in the helical region of XTcf3, Leu48 mediates binding to β-catenin via a hydrophobic pocket lined by residues Phe253 and Phe293.[77] In contrast, negatively charged residues Asp16 and Glu24 at either ends of the extended region of XTcf3-CBD interact with the positively ''charged buttons'' formed by β-catenin residues Lys312 and Lys435, respectively.[77] These residues (in the α-helix and extended region) at the β-catenin–Tcf interface are conserved in the Lef/Tcf family, suggesting a similar binding mode for all β-catenin–Tcf and β-catenin–LEF-1 interactions.

Tcf4, the human homologue of XTcf3, is predominantly expressed in human colorectal epithelial cells where it functions as an essential regulator of crypt stem cell replication and differentiation.[87] Given the shared 90% sequence identity in the N-terminal region of XTcf3 and Tcf4, it is not surprising that the structures of the human β-catenin–Tcf4 and the β-catenin–XTcf3 complex are very similar.[88] Indeed, residues 12–21 surrounding the crucial salt bridge between Tcf Asp16 and β-catenin Lys435 of both XTcf3-CBD and the Tcf4-CBD, form a nearly identical β-strand conformation. In addition, Tcf-CBD residues 41–49 of both structures form an α-helix, with Leu48 forming hydrophobic interactions with Phe253 and Phe293 of β-catenin.[77,86] Despite these similarities, several differences exist between the two structures. First, residues 1–11 are disordered in the β-catenin–Tcf4-CBD structure and do not form the β-hairpin seen in the β-catenin–XTcf3 complex.[89] Interestingly, a single conservative substitution (Asp11 in Tcf4 is a Glu in XTcf3) is responsible for this disorder.[88] Second, as observed in the β-catenin/XTcf3 structure, residues 22–29 of the XTcf3 form a β-strand extending along the groove of β-catenin, with Glu24 coordinating with β-catenin critical charged button Lys312.[89] These same residues are clustered to form a kinked α-helix in the β-catenin–Tcf4 structure. This clustering is suggested to cause the register of Tcf4 binding to shift such that Glu29, instead of Glu24 as in Tcf3, interacts with β-catenin Lys312. This shift, however, was not observed in the β-catenin–Tcf4

structure.[88] In addition, mutation studies on both Glu24 and Glu29 reduced β-catenin binding by 10–15% implying that Tcf4 can bind β-catenin Lys312 by either Glu24 or Glu29.[89,90] Finally, residues 31–37, too flexible to be resolved in the XTcf3 structure,[77] were observed to form an extended strand in human Tcf4, connecting the kinked α-helix to the C-terminal α-helix module.[89]

Although there is no apparent sequence homology between Tcf3 and C-cadherin, several β-catenin residues required for binding to XTcf3 are also required for β-catenin–cadherin complex formation, suggesting a shared β-catenin-binding domain. The β-catenin binding region of XTcf3 consists of only 60 amino acids, compared to the 100 residue binding site on cadherin, yet both XTcf3 and the cytoplasmic region of cadherin adopt similar elongated structures following the path of the β-catenin helix 3 groove.[15] In complex with β-catenin, Tcf4 residues 16–24 superimpose on the corresponding residues of XTcf3 and residues 674–682 of E-cadherin.[88] The structural positions of phosphorylated cadherin residues Ser684 and Ser686 are homologous to XTcf3 Glu26 and Glu28, which suggests that XTcf3 binding mimics that of phosphorylated cadherin.[15] From analysis of both complexes, it can be seen that the charged buttons Lys435 and Lys312, which are employed in β-catenin–Tcf3 complex formation, are also required for cadherin binding to β-catenin. The N-terminal half of the β-catenin-binding domain of C-cadherin behaves in a similar manner to the extended region of the XTcf3 β-catenin binding domain. Thus, β-catenin target binding may occur through subsites that are prone to regulatory signals (e.g. phosphorylation), which provides a possible molecular basis for β-catenin-mediated cell signalling.[15]

The β-catenin–APC complex

The role of the APC protein in tumour suppression is believed to be related to its ability to bind and downregulate β-catenin.[91,92] APC, like other β-catenin ligands, binds to the central armadillo repeat region of β-catenin through one or more of APC's four 15- and seven 20-amino acid repeats.[91,92] Several residues forming specific side chain contacts with β-catenin (Asp1022, Pro1024, Tyr1027, Tyr1031) are highly conserved in both 15mer and 20mer forms.[82] No regular secondary structure is observed in the 15mer or 20mer repeat regions of APC prior to β-catenin binding.

A comparison of the β-catenin–XTcf3 and β-catenin–E-cadherin complexes reveals that the core homology region of the first 15mer repeat (termed APC-rA) in complex with the armadillo repeat region of β-catenin binds in a manner similar to the extended regions of the other two ligands. Over the first eight residues (Leu1021–Ser1028), APC-rA adopts a near identical backbone conformation to XTcf3 (Asn15–Lys22) and E-cadherin (Tyr673–Asp680).[82] Interactions are again observed between β-catenin's charged button Lys435 and APC's Asp1022. In the APC complex, however, an alternative charged button, β-catenin Lys345 (in lieu of Lys312) forms a salt bridge with Glu1034. It should be noted that a sequence similar to the highly conserved cadherin (residues 667-684, the β-catenin-binding site) and XTcf3 β-catenin-binding sites is also observed in several of the APC 20mer repeats[12] and the homology between the N terminus of both 15 and 20mer repeats suggests a similar mechanism of β-catenin binding.[82]

The β-catenin–ICAT complex

ICAT, a negatively charged 81-residue protein, regulates the transcription of Wnt responsive genes by preventing interaction between β-catenin and the Lef/Tcf family of transcription factors.[93,94] ICAT consists of two major domains, the first being a three-helix bundle (residues 5–58), the second being an extended tail region (residues 59–75). Murine ICAT differs from human ICAT by only two residues that are not involved in ICAT–β-catenin complex formation.[95] The structure of the armadillo repeat region of β-catenin complexed with ICAT (Fig. 5) shows that binding occurs along armadillo repeats 5–12 at two distinct structural regions. First, the three-helix bundle of ICAT binds armadillo repeats 10–12 of β-catenin, anchoring ICAT to repeat 12. Second, a C-terminal tail binds armadillo repeats 5–9 along the positive groove in a manner similar to Tcf and E-cadherin. In the ICAT tail domain, Asp66 forms a salt bridge with β-catenin Lys435, and Glu75 forms a salt bridge with β-catenin Lys312;[77] these are the same charged buttons essential for E-cadherin and Tcf interaction with β-catenin.[77] Binding of the three-helical region to β-catenin is necessary and sufficient for the β-catenin–ICAT interaction, with Phe660 and Arg661 as key residues in this interaction. It is the tail region that is critical for excluding Tcf from β-catenin. This three-helix bundle provides the key interactions for "anchoring" ICAT to repeat 12 of β-catenin while the tail region "kicks" Tcf away from β-catenin via interaction with the two charged buttons (Lys312 and Lys435). This is referred to as the "anchor-and-kick" mechanism.[95]

The interaction with β-catenin's charged buttons is structurally similar for both ICAT and cadherin. Helix 1 of the ICAT three-helix bundle corresponds to a cadherin helix (residues 653–663) in the β-catenin–E-cadherin-CBD structure, despite the lack of sequence homology between the two proteins in this region. This suggests that ICAT could also interfere with β-catenin–cadherin complex formation as well as Tcf binding. Although the three-helix bundle, together with the tail of ICAT can act as a potential inhibitor of cadherin binding, it is interesting to note that although ICAT was found to effectively block the binding of Tcf to β-catenin both in vivo and in vitro, it failed to block the binding of C-cadherin to β-catenin *in vivo*.[95] The difference between these results may be due to the phosphorylation state of cadherin.

Conclusion

Biochemical and structural studies focusing on cadherin and its essential binding partners have progressed rapidly over the last decade[25] advancing our understanding of the complex mechanism of cell adhesion and associated signalling processes. Cadherin-mediated homotypic cell adhesion occurs within multiple cell types under a variety of highly diverse environmental conditions. As a result, one could argue that any of the proposed models for homophilic cadherin association may be possible depending on the surrounding cellular conditions and the varying requirements of strength and stability. Indeed, numerous dynamic changes in cadherin homophilic binding have been observed at various stages of development and neuronal networking.[28,96] Cadherin adhesion becomes even more complex as one considers the mechanism of cadherin interaction with various catenins, and their subsequent association with or dissociation from other cellular proteins. Recently, presenilin-1, a protease implicated in Alzheimer's disease, has been shown to cleave E- and N-cadherin at specific sites, thus releasing extracellular and cytoplasmic fragments of cadherins and thereby promoting disassembly of adherens junctions.[62,97] Another recent study[63] provides evidence for the involvement of the N-cadherin cytoplasmic fragment (N-cad/CTF2) in the ubiquitination of CBP, regulating CBP-dependent transcriptional activation. These events also influence β-catenin functions, as they impact on the β-catenin interactions with many target proteins such as Lef/Tcf transcription factors, APC and ICAT. Clearly, the cadherin/catenin system is a focal point of cell adhesion and intracellular signalling events. Despite these tremendous advances in our understanding of the cadherin adhesion system, many fundamental questions concerning the precise molecular mechanisms remain to be answered. First, what is the exact role(s) played by Ca^{2+} in the adhesion process? Second, why do classic cadherins share the five extracellular domain architecture, while other cadherins often contain increased numbers of the extracellular repeats? Finally, what is the precise molecular mechanism by which the extracellular event of cell adhesion controls the intracellular signalling events that lead to changes in cell shape, motility, and integrity? Further studies are needed to elucidate these and other questions concerning the biological role and function of the cadherin–catenin complex.

Acknowledgments

We would like to thank Michael B. Tropak and Rob C. Laister for their critical reading of this manuscript.

References

1. Grunwald GB. 1993. The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules. Curr Opin Cell Biol 5:797–805.
2. Alattia JR, Kurokawa H, Ikura M. 1999. Structural view of cadherin-mediated cell–cell adhesion. Cell Mol Life Sci 55:359–367.
3. Petruzzelli L, Takami M, Humes HD. 1999. Structure and function of cell adhesion molecules. Am J Med 106:467–476.
4. Edelman GM. 1984. Cell adhesion and morphogenesis: the regulator hypothesis. Proc Natl Acad Sci USA 81:1460–1464.
5. Takeichi M. 1988. The cadherins: cell–cell adhesion molecules controlling animal morphogenesis. Development 102:639–655.
6. Nose A, Takeichi M. 1986. A novel cadherin cell adhesion molecule: its expression patterns associated with implantation and organogenesis of mouse embryos. J Cell Biol 103:2649–2658.
7. Frenette PS, Wagner DD. 1996. Adhesion molecules—Part 1. N Engl J Med 334:1526–1529.
8. Humphries MJ, Newham P. 1998. The structure of cell-adhesion molecules. Trends Cell Biol 8:78–83.
9. Takeichi M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 251:1451–1455.
10. Yap AS, Brieher WM, Pruschy M, Gumbiner BM. 1997. Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function. Curr Biol 7:308–315.
11. Beavon IR. 2000. The E-cadherin–catenin complex in tumour metastasis: structure, function and regulation. Eur J Cancer 36:1607–1620.
12. Huber AH, Weis WI. 2001. The structure of the beta-catenin–E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin. Cell 105:391–402.
13. Miller JR, Hocking AM, Brown JD, Moon RT. 1999. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca^{2+} pathways. Oncogene 18:7860–7872.
14. Peifer M, Polakis P. 2000. Wnt signaling in oncogenesis and embryogenesis–a look outside the nucleus. Science 287:1606–1609.
15. Shapiro L. 2001. beta-catenin and its multiple partners: promiscuity explained. Nat Struct Biol 8:484–487.
16. Ivanov DB, Philippova MP, Tkachuk VA. 2001. Structure and functions of classical cadherins. Biochemistry (Mosc) 66:1174–1186.
17. Tepass U, Truong K, Godt D, Ikura M, Peifer M. 2000. Cadherins in embryonic and neural morphogenesis. Nat Rev Mol Cell Biol 1:91–100.
18. Vleminckx K, Kemler R. 1999. Cadherins and tissue formation: integrating adhesion and signaling. Bioessays 21:211–220.
19. Yagi T, Takeichi M. 2000. Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. Genes Dev 14:1169–1180.
20. Hill E, Broadbent ID, Chothia C, Pettitt J. 2001. Cadherin superfamily proteins in Caenorhabditis elegans and Drosophila melanogaster. J Mol Biol 305:1011–1024.
21. Truong K, Ikura M. 2002. The cadherin superfamily database. J Struct Funct Genomics 2:135–143.
22. Takeichi M. 1990. Cadherins: a molecular family important in selective cell–cell adhesion. Annu Rev Biochem 59:237–252.
23. Gumbiner B, Stevenson B, Grimaldi A. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J Cell Biol 107:1575–1587.
24. Ozawa M, Kemler R. 1990. Correct proteolytic cleavage is required for the cell adhesive function of uvomorulin. J Cell Biol 111:1645–1650.
25. Boggon TJ, Murray J, Chappuis-Flament S, Wong E, Gumbiner BM, Shapiro L. 2002. C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science 296:1308–1313.
26. 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 277:39609–39616.
27. Blaschuk OW, Sullivan R, David S, Pouliot Y. 1990. Identification of a cadherin cell adhesion recognition sequence. Dev Biol 139:227–229.
28. Takeichi M. 1995. Morphogenetic roles of classic cadherins. Curr Opin Cell Biol 7:619–627.
29. Suzuki S, Sano K, Tanihara H. 1991. Diversity of the cadherin family: evidence for eight new cadherins in nervous tissue. Cell Regul 2:261–270.
30. Tanihara H, Kido M, Obata S, Heimark RL, Davidson M, St John T, Suzuki S. 1994. Characterization of cadherin-4 and cadherin-5 reveals new aspects of cadherins. J Cell Sci 107:1697–1704.
31. Huen AC, et al. 2002. Intermediate filament-membrane attachments function synergistically with actin-dependent contacts to regulate intercellular adhesive strength. J Cell Biol 159:1005–1017.

32. Angst BD, Marcozzi C, Magee AI. 2001. The cadherin superfamily: diversity in form and function. J Cell Sci 114:629–641.
33. Norvell SM, Green KJ. 1998. Contributions of extracellular and intracellular domains of full length and chimeric cadherin molecules to junction assembly in epithelial cells. J Cell Sci 111:1305–1318.
34. Koch PJ, Franke WW. 1994. Desmosomal cadherins: another growing multigene family of adhesion molecules. Curr Opin Cell Biol 6:682–687.
35. Burdett ID. 1998. Aspects of the structure and assembly of desmosomes. Micron 29:309–328.
36. Suzuki ST. 2000. Recent progress in protocadherin research. Exp Cell Res 261:13–18.
37. Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M, Uemura T. 1999. Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell 98:585–595.
38. Chae J, Kim MJ, Goo JH, Collier S, Gubb D, Charlton J, Adler PN, Park WJ. 1999. The Drosophila tissue polarity gene starry night encodes a member of the protocadherin family. Development 126: 5421–5429.
39. Dunne J, Hanby AM, Poulsom R, Jones TA, Sheer D, Chin WG, Da SM, Zhao Q, Beverley PC, Owen MJ. 1995. Molecular cloning and tissue expression of FAT, the human homologue of the Drosophila fat gene that is located on chromosome 4q34-q35 and encodes a putative adhesion molecule. Genomics 30:207–223.
40. Ponassi M, Jacques TS, Ciani L, ffrench Constant C. 1999. Expression of the rat homologue of the Drosophila fat tumour suppressor gene. Mech Dev 80:207–212.
41. Inoue T, et al. 2001. FAT is a component of glomerular slit diaphragms. Kidney Int 59:1003–1012.
42. Cox B, Hadjantonakis AK, Collins JE, Magee AI. 2000. Cloning and expression throughout mouse development of mfat1, a homologue of the Drosophila tumour suppressor gene fat. Dev Dyn 217:233–240.
43. Ranscht B, Dours-Zimmermann MT. 1991. T-cadherin, a novel cadherin cell adhesion molecule in the nervous system lacks the conserved cytoplasmic region. Neuron 7:391–402.
44. Doyle DD, Goings GE, Upshaw-Earley J, Page E, Ranscht B, Palfrey HC. 1998. T-cadherin is a major glycophosphoinositol-anchored protein associated with noncaveolar detergent-insoluble domains of the cardiac sarcolemma. J Biol Chem 273:6937–6943.
45. Philippova MP, Bochkov VN, Stambolsky DV, Tkachuk VA, Resink TJ. 1998. T-cadherin and signal-transducing molecules co-localize in caveolin-rich membrane domains of vascular smooth muscle cells. FEBS Lett 429:207–210.
46. Overduin M, Harvey TS, Bagby S, Tong KI, Yau P, Takeichi M, Ikura M. 1995. Solution structure of the epithelial cadherin domain responsible for selective cell adhesion. Science 267:386–389.
47. Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grubel G, Legrand JF, Als-Nielsen J, Colman DR, Hendrickson WA. 1995. Structural basis of cell–cell adhesion by cadherins. Nature 374:327–337.
48. Nollet F, Kools P, van Roy F. 2000. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J Mol Biol 299:551–572.
49. Nagar B, Overduin M, Ikura M, Rini JM. 1996. Structural basis of calcium-induced E-cadherin rigidification and dimerization. Nature 380: 360–364.
50. Pertz O, Bozic D, Koch AW, Fauser C, Brancaccio A, Engel J. 1999. A new crystal structure, Ca^{2+} dependence and mutational analysis reveal molecular details of E-cadherin homoassociation. Embo J 18:1738–1747.
51. Tamura K, Shan WS, Hendrickson WA, Colman DR, Shapiro L. 1998. Structure-function analysis of cell adhesion by neural (N-) cadherin. Neuron 20:1153–1163.
52. Sivasankar S, Brieher W, Lavrik N, Gumbiner B, Leckband D. 1999. Direct molecular force measurements of multiple adhesive interactions between cadherin ectodomains. Proc Natl Acad Sci USA 96:11820–11824.
53. Sivasankar S, Gumbiner B, Leckband D. 2001. Direct measurements of multiple adhesive alignments and unbinding trajectories between cadherin extracellular domains. Biophys J 80:1758–1768.
54. Zhu B, Chappuis-Flament S, Wong E, Jensen IE, Gumbiner BM, Leckband D. 2003. Functional analysis of the structural basis of homophilic cadherin adhesion. Biophys J 84:4033–4042.
55. Ahrens T, Pertz O, Haussinger D, Fauser C, Schulthess T, Engel J. 2002. Analysis of heterophilic and homophilic interactions of cadherins using the c-Jun/c-Fos dimerization domains. J Biol Chem 277:19455–19460.
56. Chappuis-Flament S, Wong E, Hicks LD, Kay CM, Gumbiner BM. 2001. Multiple cadherin extracellular repeats mediate homophilic binding and adhesion. J Cell Biol 154:231–243.
57. He W, Cowin P, Stokes DL. 2003. Untangling desmosomal knots with electron tomography. Science 302:109–113.
58. Cepek KL, Shaw SK, Parker CM, Russell GJ, Morrow JS, Rimm DL, Brenner MB. 1994. Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. Nature 372:190–193.
59. Karecla PI, Bowden SJ, Green SJ, Kilshaw PJ. 1995. Recognition of E-cadherin on epithelial cells by the mucosal T cell integrin alpha M290 beta 7 (alpha E beta 7). Eur J Immunol 25:852–856.
60. Karecla PI, Green SJ, Bowden SJ, Coadwell J, Kilshaw PJ. 1996. Identification of a binding site for integrin alphaEbeta7 in the N-terminal domain of E-cadherin. J Biol Chem 271:30909–30915.
61. Klint P, Claesson-Welsh L. 1999. Signal transduction by fibroblast growth factor receptors. Front Biosci 4:D165–177.
62. Baki L, et al. 2001. Presenilin-1 binds cytoplasmic epithelial cadherin, inhibits cadherin/p120 association, and regulates stability and function of the cadherin/catenin adhesion complex. Proc Natl Acad Sci USA 98:2381–2386.
63. Marambaud P, et al. 2002. A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. Embo J 21:1948–1956.
64. Mengaud J, Ohayon H, Gounon P, Mege RM, Cossart P. 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of L. monocytogenes into epithelial cells. Cell 84:923–932.
65. Schubert WD, Urbanke C, Ziehm T, Beier V, Machner MP, Domann E, Wehland J, Chakraborty T, Heinz DW. 2002. Structure of internalin, a major invasion protein of Listeria monocytogenes, in complex with its human receptor E-cadherin. Cell 111:825–836.
66. Vasioukhin V, Bauer C, Yin M, Fuchs E. 2000. Directed actin polymerization is the driving force for epithelial cell–cell adhesion. Cell 100:209–219.
67. Aberle H, Schwartz H, Kemler R. 1996. Cadherin–catenin complex: protein interactions and their implications for cadherin function. J Cell Biochem 61:514–523.
68. Aberle H, Schwartz H, Hoschuetzky H, Kemler R. 1996. Single amino acid substitutions in proteins of the armadillo gene family abolish their binding to alpha-catenin. J Biol Chem 271:1520–1526.
69. McCrea PD, Turck CW, Gumbiner B. 1991. A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E-cadherin. Science 254:1359–1361.
70. Ozawa M, Nuruki K, Toyoyama H, Ohi Y. 1995. Cloning of an alternative form of plakoglobin (gamma-catenin) lacking the fourth armadillo repeat. J Biochem (Tokyo) 118:836–840.
71. Reynolds AB, Daniel J, McCrea PD, Wheelock MJ, Wu J, Zhang Z. 1994. Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. Mol Cell Biol 14:8333–8342.
72. Harrington KJ, Syrigos KN, Harington KJ. 2000. The role of E-cadherin–catenin complex: more than an intercellular glue? Ann Surg Oncol 7:783–788.
73. Nollet F, Berx G, van Roy F. 1999. The role of the E-cadherin/catenin adhesion complex in the development and progression of cancer. Mol Cell Biol Res Commun 2:77–85.
74. Ireton RC, et al. 2002. A novel role for p120 catenin in E-cadherin function. J Cell Biol 159:465–476.
75. Goodwin M, Kovacs EM, Thoreson MA, Reynolds AB, Yap AS. 2003. Minimal mutation of the cytoplasmic tail inhibits the ability of E-cadherin to activate Rac but not phosphatidylinositol 3-kinase: direct evidence of a role for cadherin-activated Rac signaling in adhesion and contact formation. J Biol Chem 278:20533–20539.
76. Stappert J, Kemler R. 1994. A short core region of E-cadherin is essential for catenin binding and is highly phosphorylated. Cell Adhes Commun 2:319–327.

77. Graham TA, Weaver C, Mao F, Kimelman D, Xu W. 2000. Crystal structure of a beta-catenin/Tcf complex. Cell 103:885–896.
78. Lickert H, Bauer A, Kemler R, Stappert J. 2000. Casein kinase II phosphorylation of E-cadherin increases E-cadherin/beta-catenin interaction and strengthens cell–cell adhesion. J Biol Chem 275:5090–5095.
79. Roura S, Miravet S, Piedra J, Garcia de Herreros A, Dunach M. 1999. Regulation of E-cadherin/Catenin association by tyrosine phosphorylation. J Biol Chem 274:36734–36740.
80. Shibamoto S, Hayakawa M, Takeuchi K, Hori T, Oku N, Miyazawa K, Kitamura N , Takeichi M, Ito F. 1994. Tyrosine phosphorylation of beta-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. Cell Adhes Commun 1:295–305.
81. Juliano RL. 2002. Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. Annu Rev Pharmacol Toxicol 42:283–323.
82. Eklof Spink K, Fridman SG, Weis WI. 2001. Molecular mechanisms of beta-catenin recognition by adenomatous polyposis coli revealed by the structure of an APC-beta-catenin complex. Embo J 20:6203–6212.
83. Kikuchi A. 1999. Roles of Axin in the Wnt signalling pathway. Cell Signal 11:777–788.
84. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. 1996. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382:638–642.
85. Provost E, Rimm DL. 1999. Controversies at the cytoplasmic face of the cadherin-based adhesion complex. Curr Opin Cell Biol 11:567–572.
86. Omer CA, Miller PJ, Diehl RE, Kral AM. 1999. Identification of Tcf4 residues involved in high-affinity beta-catenin binding. Biochem Biophys Res Commun 256:584–590.
87. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H. 1998. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 19:379–383.
88. Poy F, Lepourcelet M, Shivdasani RA, Eck MJ. 2001. Structure of a human Tcf4-beta-catenin complex. Nat Struct Biol 8:1053–1057.
89. Graham TA, Ferkey DM, Mao F, Kimelman D, Xu W. 2001. Tcf4 can specifically recognize beta-catenin using alternative conformations. Nat Struct Biol 8:1048–1052.
90. Fasolini M, Wu X, Flocco M, Trosset JY, Oppermann U, Knapp S. 2003. Hot spots in Tcf4 for the interaction with beta-catenin. J Biol Chem 278:21092–21098.
91. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P. 1993. Association of the APC gene product with beta-catenin. Science 262:1731–1734.
92. Su LK, Vogelstein B, Kinzler KW. 1993. Association of the APC tumor suppressor protein with catenins. Science 262:1734–1737.
93. Tago K, et al. 2000. Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. Genes Dev 14:1741–1749.
94. Tutter AV, Fryer CJ, Jones KA. 2001. Chromatin-specific regulation of LEF-1-beta-catenin transcription activation and inhibition in vitro. Genes Dev 15:3342–3354.
95. Graham TA, Clements WK, Kimelman D, Xu W. 2002. The crystal structure of the beta-catenin/ICAT complex reveals the inhibitory mechanism of ICAT. Mol Cell 10:563–571.
96. Bruses JL. 2000. Cadherin-mediated adhesion at the interneuronal synapse. Curr Opin Cell Biol 12:593–597.
97. Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, Robakis NK. 2003. A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. Cell 114:635–645.
98. Tsukita S, Furuse M, Itoh M. 2001. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2:285–293.
99. Overduin M, Tong KI, Kay CM, Ikura M. 1996. 1H, 15N and 13C resonance assignments and monomeric structure of the amino-terminal extracellular domain of epithelial cadherin. J Biomol NMR 7: 173–189.