CMLS, Cell. Mol. Life Sci. 61 (2004) 1884–1895
1420-682X/04/151884-12
DOI 10.1007/s00018-004-4006-2
© Birkhäuser Verlag, Basel, 2004

CMLS Cellular and Molecular Life Sciences

Review

Structure-based models of cadherin-mediated cell adhesion: the evolution continues

A. W. Koch [a,b,*], K. L. Manzur [b] and W. Shan [a]

[a] Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, Quebec H3A 2B4 (Canada)
[b] Structural Biology Program, Department of Physiology and Biophysics, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, New York 10029 (USA)

Received 7 January 2004; received after revision 24 February 2004; accepted 4 March 2004

Abstract. Cadherins are glycoproteins that are responsible for homophilic, Ca^{2+}-dependent cell-cell adhesion and play crucial roles in many cellular adhesion processes ranging from embryogenesis to the formation of neuronal circuits in the central nervous system. Many different experimental approaches have been used to unravel the molecular basis for cadherin-mediated adhesion. In particular, several high-resolution structures have provided models for cadherin-cadherin interactions that are illuminative in many respects yet contradictory in others. This review gives an overview of the structural studies of cadherins over the past decade while focusing on recent developments that reconcile some of the earlier findings.

Key words. Cell adhesion; cadherin; desmosome; cis- and trans-interactions; X-ray crystallography; nuclear magnetic resonance (NMR) spectroscopy; electron microscopy (EM); electron tomography (ET).

Introduction

Cadherins are transmembrane glycoproteins that bestow specific, homotypic adhesion properties on cells in virtually all tissues throughout the animal kingdom [1]. Cadherin-mediated cell-cell adhesion plays important roles during embryogenesis and is crucial for tissue morphogenesis and maintenance [2–4]. Cadherins are also involved in many aspects of neural development, including regionalization of the brain, neurite outgrowth and synaptogenesis [5, 6]. Defects of cadherin-mediated adhesion can often be related to tumor progression [7–9]. For example, the loss of E-cadherin function has been shown to play a causal role in the formation of carcinomas both in cultured tumor cells and in transgenic mouse models [10, 11].

* Corresponding author.
Current address: Department of Protein Chemistry, Genentech. Inc., One DNA Way, So. San Francisco, California 94080 (USA), Fax + 1 650 225 5945, e-mail: akoch@gene.com

Different classes of proteins are responsible for various mechanisms of adhesion between cells and between cells and extracellular matrix, and yet cadherins are unique in that they are responsible for homotypic, Ca^{2+}-dependent adhesion between cells. That is, in the presence of Ca^{2+}, cells expressing a certain type of cadherin only form stable contacts with cells expressing the same type of cadherin [12, 13]. Consequently, differential expression of cadherins allows cell sorting and formation of distinct tissues [14]. At the molecular level, homotypic adhesion between cells arises from homophilic interactions between cadherin extracellular domains [15, 16]. Most cadherins are single-span transmembrane glycoproteins with an extracellular region, a transmembrane helix and a single cytosolic domain. The extracellular region, also called ectodomain, has a modular structure with a variable number of extracellular cadherin domains (EC domains). While EC domains are engaged in homophilic interactions, cytosolic domains serve as a scaffold for intracellular binding partners and also link cadherins to the cytoskeleton of the cell [17].

Cadherins comprise a large superfamily of proteins that can be classified into four main subfamilies according to differences within both their extracellular regions and their cytosolic domains. These four subfamilies are classical cadherins, desmosomal cadherins, protocadherins and cadherin-like proteins (table 1) [18, 19]. Classical cadherins are found in adherens junctions and have five EC domains and a highly conserved cytoplasmic domain [17]. Cytosolic adaptor proteins named catenins interact with the cytoplasmic domain, linking classical cadherins to the cytoskeletal architecture of the cell as well as forming dynamic assemblies that are regulators of adhesiveness [20–23]. Interactions within these assemblies have been scrutinized in recent years by biochemical and structural studies, enhancing our understanding of both their architecture and regulation [24–26]. The best-characterized members of the cadherin superfamily, such as E-, N- and C-cadherin, all belong to the classical cadherins. Apart from adherens junctions, a second type of cadherin-based junction called desmosome can be found in epithelia [27]. The two kinds of desmosomal cadherins – desmogleins and desmocollins – have also five extracellular cadherin domains with significant sequence similarities to classical cadherins. Their intracellular domains, however, are distinct from each other and unrelated to the cytosolic domains from classical cadherins. The main difference between desmocollins and desmogleins is the longer cytoplasmic domain of desmogleins. The cytoplasmic domains of desmosomal cadherins are connected to the intermediate filament network via so-called plaque proteins.

Protocadherins constitute another subfamily and have mostly six or seven EC domains with lower sequence similarities to the classical cadherin group and a very distinct cytosolic domain [28]. Finally, the large group of cadherin-like proteins collectively encompasses all the proteins that have larger variations from the classical cadherin architecture, namely a larger (or in one case smaller) number of EC domains, additional non-cadherin-related domains, several transmembrane domains and/or distinct cytosolic domains [18].

New insights have been gained recently in a number of cadherin-dependent biological processes, and several excellent recent reviews have already addressed the role of cadherins during tumor progression [7, 29] and in neuronal development [30–34]. Recently published structural works, on the other hand, have provided new insights into the molecular basis for cadherin-mediated adhesion. This review will primarily focus on advances in our understanding of the molecular or even atomic details of cadherin-mediated adhesion.

Molecular basis of cell-cell adhesion mediated by cadherins

A number of approaches have been utilized to determine the molecular and structural basis for cellular adhesion events mediated by cadherins. Approaches include various cell-based studies [35–39], mutagenesis analyses [12, 40–45], immunoprecipitation followed by sedimentation analyses [46–49], electron microscopy (EM) [50–54], distance-dependent force measurements [55–57], high-resolution structural studies [X-ray and nuclear magnetic resonance (NMR)] [44, 51, 58–64], and combinations of these techniques [40, 42, 44, 51]. In addition, electron tomography of skin sections has been used very recently to unravel interactions between desmosomal cadherins in situ [65]! Several, partially contradictory models have been deduced from these works, and in the following section we will try to unravel some of the contradictions. But we will also point out similarities, and how all these studies – despite their differences – have undoubtedly greatly enlarged our understanding of cadherin-based adhesion.

Questions underlying most, if not all, attempts in understanding cadherin-mediated adhesion at a molecular level are related to determinants of cadherin specificity and to

Table 1. The cadherin superfamily.

Subfamily	Examples	Distinguishing features	Ref.
1. Classical cadherins	E-, N-, C-, R-, P-, VE-cadherins	5 EC domains; one TM domain; conserved cytosolic domains; connected to actin cytoskeleton via catenins	[13, 18]
2. Desmosomal cadherins	desmogleins; desmocollins	5 EC domains; one TM domain; cytosolic domains conserved within the two subclasses; connected to intermediate filaments via plaque proteins	[27]
3. Protocadherins	α-,β -, γ-subclasses; protocadherin-1, -11	6–7 EC domains, one TM domain; conserved cytosolic domains within subclasses; different cytosolic binding partners	[28]
4. Cadherin-like	DN-, DE-cadherin; Ret; Fat; Flamingo cadherins	Variable number of EC domains and other non-EC domains; none to several TM domains; different cytosolic domains and binding partners	[19]

E, epithelial; N, neuronal; C, compaction; P, placental; R, retinal; VE, vascular endothelial; DN, *Drosophila* neuronal; DE, *Drosophila* epithelial; EC, extracellular cadherin; TM, transmembrane.

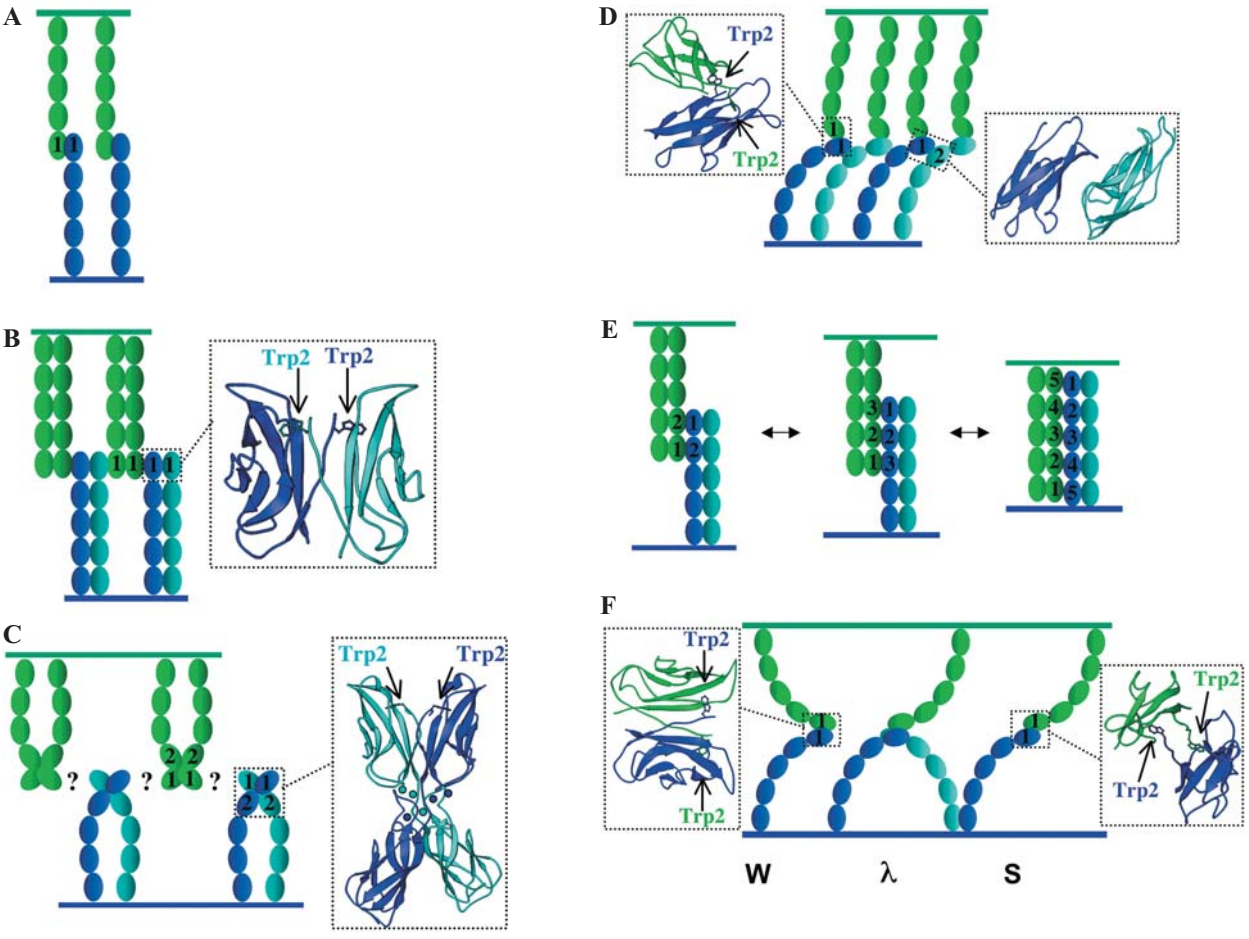

Figure 1. Evolution of structure-based cadherin models. Six principle models for cadherin interactions that evolved over the past decade are shown (*A*–*F*). Individual cadherin domains are depicted as ovals, and to better distinguish cis- from trans-interactions, cadherin ectodomains protruding from one cell surface are colored in dark or light blue, and ectodomains protruding from an opposing cell surface are shown in green. Intracellular domains and binding partners were omitted for clarity. *(A)* Prevalent model for cadherin interactions before high-resolution structures were available (see for example [1]). This model assumed an elongated shape for cadherin ectodomains and homophilic interactions between N-terminal extracellular cadherin domains 1 (EC1 domains). *(B)* Zipper model as described in the first X-ray structure of the N-terminal domain of N-cadherin (NCAD1) [61]. Two modes of homophilic interactions seen in the crystal give rise to cis-interacting strand dimers that are zipped together with strand dimers from an opposing cell surface via adhesive trans-interactions. Insert shows a ribbon representation of the Trp2-mediated cis-contact from the crystal structure. *(C)* Model for cadherin interactions derived from domain pair structures and EM studies [51, 58]. The bent rodlike shape of cadherin ectodomains as seen in EM pictures of different cadherins was taken into account, and Ca^{2+}-mediated cis-interactions were proposed from E-cadherin domain pair crystal structures. Insert: Ribbon representation of the domain pair dimer as seen in one crystal structure of E-cadherin [51]. The positions of Ca^{2+} ions are indicated by spheres. *(D)* Model for cadherin interactions from the C-cadherin crystal structure [63]. The arrangement of full-length C-cadherin ectodomains in the crystal lattice gives rise to cis- and trans-interactions. Note that the Trp2-mediated trans-contact between N-terminal domains, which is also shown as ribbon representation (left insert), is the same contact as in (*B*), only shown from a different angle. The orientation of the ectodomains, however, suggests here that the Trp2-mediated contact represents trans-interactions. Cis-interactions between EC1 and EC2 domains are shown in more detail as ribbon representation (right insert). Note that the green, opposing ectodomains also undergo cis-interactions, but these interactions were omitted here for clarity. *(E)* Model for cadherin interactions derived from distance-dependent force measurements with C-cadherin ectodomains [56]. In contrast to all other models, this model proposes trans-interactions involving more than two EC domains. *(F)* Model for cadherin interactions adapted from an ET study of a cadherin-based desmosomal junction [65]. The C-cadherin crystal structure data were fitted to electron densities obtained from ET reconstructions. As a result, interacting cadherin ectodomains give rise to three principal shapes (named W-, λ- and S-shapes), and both Trp2-mediated cis- and trans-interactions were observed. Inserts: Two Trp2-mediated trans-interactions are shown as ribbon representation. Note that these two Trp2-mediated trans-contacts represent identical interactions shown only from different angles and are virtually identical to cis-interactions from the NCAD1 crystal structure.

the role of calcium in cadherin-mediated interactions [12, 16, 59, 61]. With a growing number of cadherins having been sequenced since the late 1980s, it became apparent that at least all the members of the classical cadherins share a number of conserved features, namely a very similar domain organization (see above) and certain key residues, many of them thought to be involved Ca^{2+} binding or in adhesion [19].

Early works have pointed out the importance of Ca^{2+} for cadherins, since (i) Ca^{2+} protects cadherins against proteolysis [66]; (ii) a single point mutation of a crucial (predicted) Ca^{2+}-binding residue (Asp134 to Ala) completely abolished adhesion in cell aggregation experiments [67] and (iii) the tertiary structure of cadherins collapses in the absence of Ca^{2+} [50]. The last finding was beautifully demonstrated by EM of extracellular E-cadherin fragments, which showed not only that both recombinantly produced and biochemically isolated E-cadherin ectodomains have a bent, rodlike appearance in the presence of Ca^{2+}, but also that this rodlike structure completely collapses when Ca^{2+} is removed.

Determinants of cadherin specificity were studied by domain-swapping experiments with E-, N- and P-cadherin, and indicated early on that specificity lies in the first, most N-terminal domain (fig. 1A) [12]. In addition, peptide studies proposed that a conserved amino acid motif, which consists of the residues His, Ala and Val (HAV), and which is present in all N-terminal domains of classical cadherins, is involved in cadherin homophilic interactions [35]. One of the earliest models for cadherin-mediated adhesion, therefore, assumes an elongated shape for cadherin ectodomains and interactions exclusively between N-terminal domains (fig. 1A). Unfortunately, all these studies did not provide high-resolution data that would explain cadherin specificity at a subdomain level.

High-resolution structures of single cadherin domains

With the publication of the first high-resolution structures of cadherin domains, a plethora of data became available on cadherin interactions (table 2). The first structures published were an NMR-based structure of the N-terminal domain of E-cadherin [59] and an X-ray structure of the N-terminal domain of N-cadherin (NCAD1) [61]. Both structures demonstrated that not just these two domains, but also most likely all cadherin domains have an immunoglobulin (Ig)-like, seven-stranded β-sandwich topology with Ca^{2+} binding sites located at the bottom – or C-terminal end – of the sandwich. In addition, the X-ray structure of NCAD1 provided a compelling model for cadherin interactions (fig. 1B). Two distinct interfaces were observed in the crystal, and were thought to reflect two different kinds of interactions in vivo. One interface was a parallel, cis-interface (also called strand-dimer interface), which seemed to represent a lateral contact between cadherins on the same cell surface. The second interface, a so-called antiparallel, trans-contact, was believed to reflect the adhesive contact between cadherin molecules emerging from opposing cells. Remarkably, the former interface involved the mutual insertion of the side chain of a conserved tryptophan residue (Trp2) into a conserved hydrophobic pocket of a partner molecule. This 'exchange' of Trp side chains between two partner molecules is undoubtedly the most prominent feature of this interface, but residues of β-strand A that follow Trp2 are involved in interactions as well, creating a very intimate contact between two cadherin domains. The importance of this Trp-mediated contact for the overall process of adhesion was subsequently confirmed by several point mutations in combination with cell adhesion assays [42, 44]. The trans-contact, on the other hand, engages a number of residues from β-strands C, D, F and G and from a so-called quasi β helix, which is unique to N-terminal cadherin domains. Residues involved in these interactions also included the HAV motif on β-strand F, but only histidine and valine appeared to take part in trans-interactions, whereas the alanine side chain is buried within the hydrophobic core.

Although, it is sometimes difficult to interpret the relevance of crystal contacts in terms of in vivo protein-protein interactions, the authors point out that the same crystal contacts were observed in several different crystal forms and that the covered surface areas for both contacts

Table 2. Overview of important high- to medium-resolution structures of cadherins.

Name	Type of cadherin	Recombinant construct (extra residues in parentheses)	Technique	Ref.
ECAD1	mouse E-cadherin	(Met-Arg-)Asp1-Trp2 … Ala144	NMR spectroscopy	[59]
NCAD1	mouse N-cadherin	(Gly-Ser-)Asp1-Trp2 … Phe108	X-ray crystallography	[61]
ECAD12	mouse E-cadherin	(Met-Arg-)Asp1-Trp2 … Asp213	X-ray crystallography	[58]
NCAD12	mouse N-cadherin	Asp1-Trp2 … Phe223	X-ray crystallography	[44]
ECAD12	mouse E-cadherin	(Met-)Asp1-Trp2 … Pro219	X-ray crystallography	[51]
CCAD15	Xenopus C-cadherin	Asp1-Trp2 … Lys542	X-ray crystallography	[63]
Desmosomal junction	mouse desmosomal cadherins	n.a.	electron tomography	[65]

E, epithelial; N, neuronal; C, compaction; NMR, nuclear magnetic resonance.

were too large to be considered artificial crystal-packing contacts. A model for cadherin interactions deduced from this crystal structure also assumed that domains 2–5 would undergo similar cis-interactions, creating tightly interwoven strand-dimers that would be zipped together with dimers from an opposing cell via trans-interactions (fig. 1B). This so-called zipper model drew a lot of attention, since it appeared to readily explain how cadherins interact at a molecular or even atomic level. Unfortunately, some aspects of this model could not be confirmed by later structural and cell biology-based studies.

As mentioned earlier, an NMR-based structure of E-cadherin [59], which was published around the same time as the NCAD1 structure, showed that the N-terminal domain of E-cadherin has a very similar topology to NCAD1. However, unlike the X-ray structure, this NMR-based structure did not provide a model for cadherin homophilic interactions, as the recombinant fragment used for NMR experiments was monomeric in solution and no domain-domain interactions were observed. The authors of this study nevertheless suggested that residues of a concave surface, made up of residues of β-strands C, F and G, might be involved in homophilic interactions, but that other domains could be involved as well, given the monomeric state of the N-terminal domain in this NMR study. It should also be pointed out that the E-cadherin fragment used for the NMR study was considerably larger than the N-cadherin fragment of the crystal structure and comprised almost one and a half domains (table 2) [68]. Approximately 40 residues C-terminal to the folded N-terminal domain were consequently disordered in the NMR structure, and it is possible that these 'extra' residues prevented dimer formation.

Despite this disparity concerning interactions, both the NMR and crystal structures provided first insights into the molecular details of Ca^{2+} binding to cadherins. The position of heavy metal atoms in the crystal structure at the bottom of the roughly barrel-shaped cadherin domain indicated the location of Ca^{2+} binding sites, but the NMR structure provided a more detailed picture, since chemical shift changes upon Ca^{2+} binding helped to pinpoint residues directly or indirectly involved in Ca^{2+} binding.

Two-domain structures and the role of Ca^{2+}

The third published high-resolution cadherin structure utilized a two-domain fragment of E-cadherin (ECAD12, table 2) and confirmed that Ca^{2+} binding sites are located at the bottom of a cadherin barrel, exactly between two cadherin domains [58]. This crystal structure revealed that residues from the linker region connecting two cadherin domains and residues from both domains generate a network of interactions with three Ca^{2+} ions. The ECAD12 structure, therefore, helped us to understand the earlier discovered importance of Ca^{2+} for the rodlike structure and for the trypsin resistance of cadherin ectodomains, since Ca^{2+} binding in between consecutive domains stabilizes otherwise flexible linker regions. The details of Ca^{2+} binding seen in the ECAD12 structure also explains the dramatic effect on cadherin-mediated adhesion caused by mutating an aspartate residue (Asp134), as this point mutation eliminates an essential Ca^{2+}-binding residue [67].

EM studies with oligomerized E-cadherin ectodomains provided additional details for cadherin-cadherin interactions [51, 54]. In these studies E-cadherin ectodomains were recombinantly fused to the pentamerizing coiled-coil domain of COMP (cartilage oligomeric matrix protein) to create a high local concentration of cadherin domains. While the resolution of these studies was not high enough to evaluate interactions at the subdomain level, it supported the notion that cadherins interact via their most N-terminal domains. Moreover, these studies showed that cadherins have to first undergo cis-interactions in order to be able to subsequently form trans-interactions. Later, we were able to demonstrate that distinct Ca^{2+} concentrations are required during each step of this two-step process, further corroborating the significance of such a mechanism [51]. Trans-interactions seemed to require physiological Ca^{2+} concentrations (1–2 mM), whereas cis-interactions occurred already at much lower Ca^{2+} levels (approximately 0.5 mM), and even lower Ca^{2+} concentrations (~0.05 mM) were sufficient to support the rodlike shape of E-cadherin ectodomains. The requirement of high Ca^{2+} concentrations for trans-interactions between N-terminal cadherin domains also supported the earlier suggested existence of low-affinity Ca^{2+} binding sites in close proximity to the N-terminal domain of E-cadherin [69]. A recent detailed NMR spectroscopy-based study also demonstrated an effect of Ca^{2+} on dimerization, and possibly on higher-order aggregation, and stressed the requirement of both high Ca^{2+} and high protein concentrations for cadherin interactions beyond dimer formation [64].

Conflicting models for homophilic interactions

The electron microscopy-based evidence of a two-step process for cadherin interactions has been further supported by a number of cell-based studies [36, 39, 47, 70, 71]. In particular, the formation of cis-dimers was shown by chemical crosslinking of cadherins on cell surfaces [38] and by co-immunoprecipitation assays followed by sedimentation analysis [46–48]. In contrast to the ECADCOMP studies mentioned above, some studies also suggested Ca^{2+}-independent, cis-dimer formation [36, 47]. Further evidence for the existence of cis-dimers came from studies with cultured hippocampal neurons, where synaptic stimulation leads not only to formation of N-cadherin dimers but also to enhanced protease resis-

tance [72]. In addition, this work also stressed the importance of cis-dimers for N-cadherin mediated synaptic junctions. Dimer formation also seems to be crucial for the less frequently observed, heterophilic interactions between cadherins, since Ca^{2+}-dependent cis-heterodimers between N- and R-cadherin were observed in L-cell transfection assays [42].

Consequently, a cis-dimer that was seen in the already mentioned crystal structure of ECAD12 was assumed to represent the functional cis-dimers on cell surfaces [58]. But as elucidating as the ECAD12 structure was regarding the role of Ca^{2+} (see above), it did not provide a satisfying model for homophilic interactions of cadherins, for two main reasons. First, none of the crystal contacts seemed to reflect adhesive, trans-interactions, and second, the cis-interactions contradicted the N-cadherin 'zipper model'. The ECAD12 structure showed two molecules forming an intertwisted X-shaped dimer that are closest together at their Ca^{2+} binding sites (fig. 1C). However, this contact is by far not as pronounced as the one between two NCAD1 molecules in the earlier crystal structure (fig. 1B), as the ECAD12 cis-contact is mainly formed by water-mediated hydrogen bonds. The conserved Trp residue, which mediates the cis-contact in the NCAD1 structure, is not even resolved in the ECAD12 structure, and given the geometry of the dimer arrangement, it seemed unlikely that this residue could be engaged in a similar interface as observed in NCAD1.

Interestingly, a second N-cadherin structure, comprising the two N-terminal N-cadherin domains (NCAD12, table 2), is virtually identical to the ECAD12 structure and also falls short of substantiating the zipper-model, as both contacts that gave rise to the zipper are absent in the NCAD12 structure as well [44]. Similar to the ECAD12 structure, the crucial Trp2 is disordered, and a solvent-exposed hydrophobic pocket is visible. In addition, it was demonstrated in the same study that mutating Trp2, or residues that form the hydrophobic pocket, completely abolishes adhesion. Since these mutations, however, were analyzed by cell aggregation assays, it was impossible to determine whether cis- or trans-interactions were abolished. Nonetheless, this work demonstrated the importance of Trp2 itself and the hydrophobic pocket for the overall process of cadherin-mediated adhesion.

Another work, including another X-ray structure of an almost identical two domain fragment of E-cadherin (table 2), was set up to solve some of the contradictions described above [51]. This second ECAD12 structure was almost identical to the earlier one, but here the Trp2 side chain was visible and clearly buried into the hydrophobic pocket of its own domain and not of a partner molecule (fig. 1C). Similar to the first ECAD12 structure, this crystal structure also failed to offer a model for trans-interactions. Only the bound state of the Trp2 side chain was confirmed as a prerequisite for trans-interactions, since in the same work the ECADCOMP system described above was used to demonstrate that mutating Trp2 abolished trans-interactions, but not cis [51]! Therefore, an alternative model for cadherin interactions was proposed, suggesting the formation of Ca^{2+}-mediated cis-dimers and an indirect role of Trp2 in trans-interactions (fig. 1C).

This Trp2-dependence of trans- but not cis-interactions was further corroborated by EM studies of cadherin Jun/Fos heterodimers [52]. Heterodimers were created by recombinantly fusing cadherin ectodomains with the leucine zipper domains of c-Jun and c-Fos, which are known to predominantly form heterodimers. EM of E-cadherin Jun/Fos dimers showed that a Trp2Ala point mutation in one E-cadherin ectodomain abolishes trans-interactions between two dimers but not cis-interactions within a dimer. The same EM studies also demonstrated that specificity for homophilic interactions already exists on the level of cis-dimers, since neither cis- nor trans-interactions were observed with a Jun/Fos heterodimer of E- and P-cadherin ectodomains [52].

Given the variable state of Trp2 in different structures, a switch mechanism was proposed between a nonactive state with solvent-exposed hydrophobic pocket and an active, presumably dimeric state with hydrophobic pockets filled by Trp side chains [44].

A possible explanation regarding the different contacts observed in the different crystal structures was originally sought in small, but potentially crucial differences within the construct [16, 63]. In most of the structural works, the importance of N-terminal residues for the function of cadherins was largely overlooked. Mainly as a consequence of cloning artifacts, all but one out of five constructs used for structure solution had additional N-terminal residues (table 2), although it had been shown much earlier that a few extra residues prior to the first residue of domain 1, which is a conserved Asp in classical cadherins, can abolish cell adhesion [83]. However, despite their different N-termini, the three two-domain structures are all fairly similar, with the main difference being the disordered or bound state of the Trp2 side chain, and the only structure with the correct N-terminus – NCAD12 – provided a compelling model neither for trans-interactions nor cis-interactions [44]. Conversely, all three domain pair structures differ from the NCAD1 structures with regards to cis- and trans-interactions. Therefore, although additional residues at the N-terminus might effect trans-interactions, the main reason for differences between the NCAD1 structure and the two-domain structures might simply be sought in the fact that they comprise a different number of domains. Ironically, the larger two-domain constructs, which on the one hand nicely explain the function of Ca^{2+}, are seemingly less elucidating concerning trans-interactions. With the information available up to that point, only a general impor-

tance of Trp2 and its surrounding residues could be confirmed, since a number of independent studies clearly demonstrated the loss of cadherin function after mutating Trp2 itself or residues that form the hydrophobic pocket in which Trp inserts its side chain [42, 44, 73].

Weakness of cadherin interactions

Another reason for observing different interactions in the various crystal structures can be sought in the intrinsic weakness of cadherin homophilic interactions. There are now several lines of evidence suggesting that interactions between single cadherin molecules are rather weak and that only the high local concentration of cadherins on cells leads to a strong adhesive contact, probably due to an increase in avidity [36, 38, 54]. This was supported by the observation that cadherin interactions are difficult to detect in solution, but can be observed after clustering of cadherin ectodomains (see below). In vivo, extracellular domains [39], cytoplasmic regions [71, 74] and also transmembrane domains [75] all contribute to or influence lateral clustering of cadherins. Therefore, to mimic the cluster formation of cadherins naturally occurring on cell surfaces, cadherin ectodomains were recombinantly fused with the oligomerization domains from COMP [51, 54], CMP (cartilage matrix protein) [53] and Jun/Fos [52], or with the Fc fragment of antibodies [76]. Interactions within or between cadherin clusters – e.g. dimers, trimers or pentamers – could then be observed in solution and were used as a model to describe cadherin interaction on cells. In a similar approach, C-cadherin was clustered directly on cells by replacing its cytosolic domain with a protein domain that dimerizes upon addition of a small organic compound, and thereby the effect of cadherin clustering could be scrutinized independently from cytosolic interactions [39]. Despite the replacement of their cytosolic domains, these artificially clustered cadherins were able to strengthen intercellular adhesion and therefore presumably able to undergo trans-interactions.

Direct measurements of dissociation constants (K_Ds) between cadherin domains by sedimentation analysis with analytical ultracentrifugation also point to weak interactions. For the dimerization of the E-cadherin two-domain fragment, for example, apparent K_Ds of 0.8 mM [69] and 0.2 mM [77], respectively, have been determined. The dissociation constant determined for the entire C-cadherin ectodomain was significantly smaller (0.06 mM), but still indicated weak binding [40]. Of course, it cannot be judged from these sedimentation analysis experiments whether the observed dimers are cis- or trans-dimers, but – as outlined above – the notion that cis-interactions are a prerequisite for trans-interactions had already been widely accepted. Notably, in both cases no higher oligomeric species were detected, leading to the conclusion that cadherin trans-interactions are even weaker. Trans-interactions between vascular endothelial (VE)-cadherin dimers were characterized by atomic force microscopy and indeed suggested low-affinity binding (K_D = 0.01 – 1 mM) for this cadherin [78].

In general, crystal-packing constraints can influence protein-protein interactions during the crystallization process, especially when these interactions are weak. Consequently, nonphysiological crystal-packing forces might become the main driving force for the formation of protein interactions. In addition, it has to be reiterated that all the cadherin crystal structures described so far only included a part of an extracellular region of a transmembrane protein that in turn is part of a multiprotein complex. In adherens junctions, cadherin ectodomains constitute only the extracellular part of the cadherin-catenin complex, and it is important to note that major parts of this protein complex are already assembled before cadherins are exported to the cell surface [79]. The major restraint concerning cadherin homophilic cis-interactions in vivo, therefore, might be the oriented clustering of cadherins on cell surfaces, achieved through linkage to the cytoskeleton. In addition, the rather rigid rod-like structure of cadherin ectodomains due to Ca^{2+} binding might also restrict possible interactions. Thus, cadherins might be predisposed to undergo certain specific interactions in vivo that might not be favored during some experiments in solution, in particular during crystallization of isolated cadherin domains.

High-resolution structure of an entire cadherin ectodomain

To resolve the possible problem of dealing only with parts of a cadherin ectodomain, the entire extracellular region of a representative classical cadherin – C-cadherin – was crystallized, and the structure was solved at a resolution of 3.1Å [63]. In comparison with the NCAD1 structure [61], the C-cadherin structure refutes the earlier proposed adhesive trans-interface, reinterprets the cis-interface as trans-interface, and suggests a completely different cis-contact.

In the C-cadherin structure, virtually the same Trp2-mediated crystal contact was observed as in the NCAD1 structure; however, a completely different model for cis- and trans-interactions was proposed. In the NCAD1 structure, the Trp-mediated contact was interpreted as a cis-interaction because the C-termini of partner molecules were pointing in similar directions, and because of the presence of a second contact, in which partner molecules were clearly aligned in opposite directions. The cis-interaction was then coined strand-dimer interaction since it was assumed that all the other cadherin domains of two partner molecules would undergo the same kind of interactions. Moreover, the formation of functional dimers was also observed in cell-based studies, and these

dimers were widely considered as equivalent to the strand-dimers. In the C-cadherin structure, however, the entire ectodomain has a curved shape similar to the one observed earlier by EM of the ectodomain of E-cadherin [50]. Furthermore, ectodomains are clearly arranged in opposite directions, as if protruding from opposing cell surfaces (fig. 1D). Consequently, the Trp-mediated strand-dimer interface was interpreted here as the adhesive trans-contact. The authors maintain the term 'strand-dimer' for the Trp-mediated trans-contact, which might lead to some confusion, especially in light of cell-based studies, since it implies a parallel, cis-contact between molecules from the same cell surface.

One of the compelling results of the C-cadherin structure, besides the fact that it provides, for the first time, a high-resolution picture of an entire extracellular cadherin domain, is that it also reconciles some of the earlier findings concerning cadherin interactions. First, the C-cadherin structure explains adequately why adhesive trans-interactions were lost upon mutating Trp2, or residues of its binding pocket, as it was demonstrated earlier, for example by EM of clustered E-cadherin ectodomains [51, 52]. Second, the C-cadherin structure also provides a model for cis-interactions, since a second interface was observed in this crystal structure, in which residues of a concave surface of EC1 interact in cis with residues of a complementary convex surface of EC2 of a partner molecule (fig. 1D). Since this interaction surface also includes residues of the linker region between EC2 and EC3, the authors speculate that this contact further explains results from another study, which showed three EC domains are necessary for full adhesive function [40]. Interestingly, a similar cis-contact is also present in the two earlier ECAD12 structures [63], but it was not commented on, since it may have been interpreted as a non-physiological crystal contact. A similar Ca^{2+}-mediated cis-dimer as seen in the ECAD12 structures is not present in the C-cadherin structure, but Ca^{2+} binding could still affect cis-interactions, as the cis-contact observed in the C-cadherin structure also seemed to involve residues of the Ca^{2+}-binding linker region.

Neither the C-cadherin structure nor site-directed mutagenesis experiments could confirm the earlier trans-contact from the NCAD1 structure. Therefore, the NCAD1 trans-contact is now considered a crystal artifact, reflecting earlier discussed difficulties in predicting physiological protein interactions from crystal contacts. The Trp-mediated contact that is now considered a trans-contact, on the other hand, seems to be physiologically relevant and was supported by a number of independent studies, such as site-directed mutagenesis studies in combination with cell adhesion assays [42, 44].

The main caveat of the C-cadherin structure is, however, that the adhesive trans-interface seen in this crystal structure does not provide a compelling model for the homophilic nature of cadherin-cadherin interactions, and, therefore, does not explain cadherin specificity. Most of the residues involved in trans-interactions are conserved among classical and desmosomal cadherins, and can consequently not confer specificity to cadherins. Nevertheless, a few residues engaged in trans-interactions are not conserved, and could, hence, contribute to subtle differences in binding affinities between individual cadherin domains that ultimately amount to significant differences in binding affinities at intercellular junctions. On the other hand, the Trp-mediated trans-contact explains the emerging view that cadherins are more versatile in their interactions with other cadherins as previously assumed [80], i.e. cadherin interactions are not always strictly homophilic. Heterophilic interactions have been observed between different cadherins, such as N- and R-cadherin [42], or between cadherins and other adhesion molecules such as integrins [81, 82].

Ultimately, all structure-derived models about cadherin interactions have to be confirmed by a rigorous mutational analysis in conjunction with a functional evaluation of mutated proteins. So far, convincing site-directed mutagenesis data only addressed residues of the Trp2-mediated contact [42, 44, 47, 51, 73], Ca^{2+}-binding residues [63] and residues that are important for the structural integrity of cadherin domains [41]. These existing mutational data are largely in agreement with the C-cadherin structure, but it remains to be seen whether this new model can inspire other site-directed mutagenesis experiments that would also explain cadherin specificity.

Despite the fact that all the structural works were unable to adequately explain cadherin specificity, they all agreed upon the assumption that specificity lies in the most N-terminal part of cadherins. This was proposed for the first time in domain-swapping experiments [12] and has since then been supported by a number of cell-based studies [37, 48, 49], and all the high-resolution structural studies and EM studies mentioned above. While all the models mentioned so far stress the importance of the most N-terminal domains for trans-interactions, there is also an alternative model that suggests that more than just one or two domains are engaged in trans-interactions. This model has been derived from surface force measurements with C-cadherin ectodomains [84] and was also supported by bead aggregation assays, which implied that at least domains EC1 through EC3 are required for homophilic binding activity, whereas EC3 could be replaced by EC4 or EC5. Distance-dependent surface force measurements with C-cadherin ectodomains suggested that cadherins interact not only via their most N-terminal domains, but can undergo several modes of interactions, including one, several or even all five extracellular domains (fig. 1E). The latter would also allow a complete interdigitated association of the five extracellular domains (fig. 1E) [55–57]. None of these models can ultimately

be rebutted or confirmed entirely, and the question remains which model is the most physiologically relevant.

Electron tomography of cadherin-based junctions

Structural studies with entire ectodomains more closely resemble the in vivo state than studies with single domains or domain pairs, but it has also been shown in cell-based studies that cadherins without linkage to the cytoskeleton confer only weak adhesiveness and that cytosolic domains and their binding partners are required to establish strong and functional adhesive contacts. A new study using electron tomography (ET) has overcome the limitation of studying only fragments of cadherin molecules rather than an entire adhesive complex [65].

ET is a noninvasive three-dimensional (3D) imaging technique and was used to visualize entire desmosomal junctions in situ. For ET analysis, conventional plastic sections of epidermis from newborn mice were prepared after high-pressure freezing and freeze substitution to preserve delicate structures of desmosomes such as membranes and cytoskeletal filaments. Dual axis tilt series of images were recorded to achieve a more isotropic resolution and lower background noise. After 3D reconstruction of desmosomal junctions from image tilt series, densities were manually attributed to particular protein components. The principle architecture of desmosomal junctions is well known, and in ultrathin sections of skin three major desmosomal substructures can be distinguished, namely intermediate filaments, plaque proteins and EC domains. In the thicker sections used for ET, the plasma membrane is also clearly visible as a white, 3.5-nm-wide space paralleled by two black lines. The fingerlike structures that cross a 28-nm-wide intercellular space in the tomographic reconstruction, therefore, have to be attributed to the extracellular domains of desmosomal cadherins. Electron dense knots along the midline of the intercellular space indicate interaction points between different molecules. These fingerlike structures – and the interactions along the midline – are not arranged in a regular pattern as seen in the crystal structure of C-cadherin, but their curved shapes resemble the structure of C-cadherin ectodomains [63] and the EM images of E-cadherin ectodomains [54]. To dissect interactions between individual molecules seen in 3D reconstructions of desmosomes, the authors use the C-cadherin crystal structure [63] to fit high-resolution X-ray data to individual densities. The high degree of sequence similarity between classical cadherins and desmosomal cadherins was quoted to justify this approach.

The authors examined all the densities in one single junction and detected three different geometries for interacting cadherin molecules, which they termed W-, S- or λ-shapes (fig. 1F). By fitting the C-cadherin X-ray structure to these shapes, it became apparent that the W-shapes are consistent with Trp2-mediated trans-dimer as seen in the C-cadherin X-ray structure. The formation of W-shaped dimers, therefore, would be triggered by mutual insertions of two Trp side chains in the hydrophobic pocket of partner molecules. S-shapes can be described as trans-dimers in which one molecule is rotated by about 90° compared with W-shaped dimers. In S-shaped dimers, therefore, one Trp2 would be pulled out of the pocket and would be available – together with its now empty acceptor pocket – for additional interactions. In this way interactions are propagated, and λ-shapes can be described as S-shapes with an additional Trp2-mediated cis-interaction. Ultimately, of course, more than just Trp2 and residues of the conserved hydrophobic pocket are involved in interactions. A model for assembly, therefore, was proposed in which a recognition step involving Trp2 and its hydrophobic pocket is followed by a compaction step, resulting in a network of interactions that lead to higher adhesive strength.

One immediate and interesting result of this ET study is that desmosomal junctions do not display a regular lattice of molecules and interactions, as would have been expected from crystal structures. Instead, discrete groups of four to six molecules are found that can be described as a combination of the W-, S- and λ-shapes described above (fig. 1F). In addition, only tip-to-tip interactions were observed, and after fitting individual C-cadherin molecules to observed densities, no EC1-EC2 interactions seem plausible (fig. 1F). Clearly, the ET images also contradict an interdigitation of several or all five cadherin domains. It should also be mentioned that this work also offers an explanation for the crucial role of EC5 in adhesion. Several of these membrane proximal cadherin domains form pairs or triplets with other EC5 domains. Interestingly, most of the EC5 pairs are engaged in interactions with different cadherin molecules via their N-terminal domains. Therefore, these EC5-EC5 interactions add another level of interaction and explain, for example, why antibodies or mutations interfering with EC5 have such dramatic effects for cadherin-mediated adhesion.

Altogether, this work stresses once more the crucial role of Trp2 and its hydrophobic acceptor pocket by suggesting that Trp can mediate both cis- and trans-interactions. Each of the earlier crystal structures, therefore, might just have depicted one of several possible Trp-mediated interfaces. Therefore, this recent ET study reconciles some of the earlier findings while also stressing the flexibility of intermolecular interfaces in cadherin interactions.

Despite the similarities between classical cadherins and desmosomal cadherins, it would be interesting to visualize cadherin-based adherens junctions by ET reconstructions. But there are a few reasons why this might be a more difficult endeavor as compared with the ET of desmosomes. Some reasons might be simply due to technical limitations. For example, for study of desmosomal

junctions, mouse epidermis could be used, which is rich in desmosomes and easier to handle than probably most of the tissues rich in adherens junctions. Desmosomal junctions are also easier to recognize than adherens junctions, mainly because of their dense plaque structure adjacent to the intracellular side of the plasma membrane. Finally, desmosomes are more uniform in the intercellular space, as they consist mainly of the two types of desmosomal cadherins. The only densities that could not be accounted for in the ET study of desmosomes was found near the membrane surface and was attributed to a 128-amino acid, glycosylphosphatidiylinositol (GPI)-anchored protein that was reported to copurify with desmosomes. In contrast to desmosomes, adherens junctions display a more complicated molecular architecture in the intercellular space and often contain other non-cadherin proteins, for example nectin, for which no structural information is available [85].

Conclusion

Almost a decade after the first crystal structure of a cadherin domain was published, titled 'Structural basis of cell-cell adhesion by cadherins' [61], we still have much to learn about the details of the molecular mechanisms underlying cadherin-mediated adhesion. The intrinsic weakness of cadherin-cadherin interactions and the – now relatively undisputed – fact that cadherins undergo two kinds of homophilic interactions undoubtedly contributed to difficulties in unraveling these interactions at a molecular or even atomic level. It is possible that many of the different interactions observed are neither correct nor incorrect, but that a particular experimental setting simply favors certain interactions.

Attempts to scrutinize the exact role of a conserved Trp residue exposed the difficulties in interpreting cadherin interactions. In the single domain structure of N-cadherin, Trp2 and its acceptor pocket are exclusively engaged in domain-domain interactions. In three different two-domain structures, Ca^{2+} binding causes a straight, nonflexible conformation of the cadherin molecule, and it is therefore conceivable that in these constructs, crystal contacts at the Ca^{2+}-binding regions are energetically favored over Trp-mediated contacts. Consequently, in neither of the domain pair structures were Trp side chains found to be engaged in domain-domain interactions. The curved shape of an entire ectodomain, as seen in the C-cadherin structure, might render Ca^{2+}-mediated contacts less probable. Trp-mediated interactions, however, are possible and were observed as trans-interactions. Finally, in the ET study of an entire junction, Trp was found to be engaged in both cis- and trans-interactions, reconciling earlier observations and clearly pointing out the versatility of this mechanism for cadherin interactions. Since the importance of Trp-mediated interactions was also confirmed by a number of cell-based studies, including mutagenesis studies, it is now widely accepted as a crucial mechanism for cadherin-mediated adhesion.

Trp2 and most of the residues of its acceptor pocket are conserved among all classical and desmosomal cadherins, and hence this mechanism does not provide a model for the homophilic nature of cadherin interactions. Though the intrinsic weakness of cadherin interactions might explain the difficulties in detecting subtle differences in interactions that could ultimately confer specificity to cadherins, the question still remains what determines homophilic specificity.

Acknowledgments. We are grateful to Dr David R. Colman and Dr Ming-Ming Zhou for their continuous support. A.W.K. was supported by grants from the National Institutes of Health (NS20147) and from the National Multiple Sclerosis Society (RG3217), K.L.M. was supported by a grant from the National Institutes of Health (CA87658) and W.S. was supported by a grant from the National Institutes of Health (NS41687).

1 Takeichi M. (1990) Cadherins: a molecular family important in selective cell-cell adhesion. Annu. Rev. Biochem. **59:** 237–252
2 Gumbiner B. M. (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell **84:** 345–357
3 Takeichi M. (1995) Morphogenetic roles of classic cadherins. Curr. Opin. Cell Biol. **7:** 619–627
4 Tepass U., Truong K., Godt D., Ikura M. and Peifer M. (2000) Cadherins in embryonic and neural morphogenesis. Nat. Rev. Mol. Cell. Biol. **1:** 91–100
5 Ranscht B. (2000) Cadherins: molecular codes for axon guidance and synapse formation. Int. J. Dev. Neurosci. **18:** 643–651
6 Redies C. (2000) Cadherins in the central nervous system. Prog. Neurobiol. **61:** 611–648
7 Christofori G. (2003) Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression. EMBO J. **22:** 2318–2323
8 Van Aken E., De Wever O., Correia da Rocha A. S. and Mareel M. (2001) Defective E-cadherin/catenin complexes in human cancer. Virchows Arch. **439:** 725–751
9 Behrens J. (1999) Cadherins and catenins: role in signal transduction and tumor progression. Cancer Metastasis Rev. **18:** 15–30
10 Christofori G. and Semb H. (1999) The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. Trends Biochem. Sci. **24:** 73–76
11 Perl A. K., Wilgenbus P., Dahl U., Semb H. and Christofori G. (1998) A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature **392:** 190–193
12 Nose A., Tsuji K. and Takeichi M. (1990) Localization of specificity determining sites in cadherin cell adhesion molecules. Cell **61:** 147–155
13 Kemler R. (1992) Classical cadherins. Semin. Cell Biol. **3:** 149–155
14 Takeichi M., Hatta K., Nose A., Nagafuchi A. and Matsunaga M. (1989) Cadherin-mediated specific cell adhesion and animal morphogenesis. Ciba Found. Symp. **144:** 243–249
15 Yap A. S., Brieher W. M. and Gumbiner B. M. (1997) Molecular and functional analysis of cadherin-based adherens junctions. Annu. Rev. Cell. Dev. Biol. **13:** 119–146
16 Koch A. W., Bozic D., Pertz O. and Engel J. (1999) Homophilic adhesion by cadherins. Curr. Opin. Struct. Biol. **9:** 275–281

17 Aberle H., Schwartz H. and Kemler R. (1996) Cadherin-catenin complex: protein interactions and their implications for cadherin function. J. Cell. Biochem. **61:** 514–523
18 Angst B. D., Marcozzi C. and Magee A. I. (2001) The cadherin superfamily: diversity in form and function. J. Cell Sci. **114:** 629–641
19 Nollet F., Kools P. and 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
20 Gumbiner B. M. (2000) Regulation of cadherin adhesive activity. J. Cell Biol. **148:** 399–404
21 Lilien J., Balsamo J., Arregui C. and Xu G. (2002) Turn-off, drop-out: functional state switching of cadherins. Dev. Dyn. **224:** 18–29
22 Steinberg M. S. and McNutt P. M. (1999) Cadherins and their connections: adhesion junctions have broader functions. Curr. Opin. Cell Biol. **11:** 554–560
23 Yap A. S. and Kovacs E. M. (2003) Direct cadherin-activated cell signaling: a view from the plasma membrane. J. Cell Biol. **160:** 11–16
24 Pokutta S., Drees F., Takai Y., Nelson W. J. and Weis W. I. (2002) Biochemical and structural definition of the l-afadin- and actin-binding sites of alpha-catenin. J. Biol. Chem. **277:** 18868–18874
25 Huber A. H. and Weis W. I. (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
26 Pokutta S. and Weis W. I. (2000) Structure of the dimerization and beta-catenin-binding region of alpha-catenin. Mol. Cell. **5:** 533–543
27 Huber O. (2003) Structure and function of desmosomal proteins and their role in development and disease. Cell. Mol. Life Sci. **60:** 1872–1890
28 Frank M. and Kemler R. (2002) Protocadherins. Curr. Opin. Cell Biol. **14:** 557–562
29 Mareel M. and Leroy A. (2003) Clinical, cellular and molecular aspects of cancer invasion. Physiol. Rev. **83:** 337–376
30 Dustin M. L. and Colman D. R. (2002) Neural and immunological synaptic relations. Science **298:** 785–789
31 Goda Y. (2002) Cadherins communicate structural plasticity of presynaptic and postsynaptic terminals. Neuron **35:** 1–3
32 Guthrie S. (2002) Neuronal development: sorting out motor neurons. Curr. Biol. **12:** R488–R490
33 Huntley G. W., Gil O. and Bozdagi O. (2002) The cadherin family of cell adhesion molecules: multiple roles in synaptic plasticity. Neuroscientist **8:** 221–233
34 Yagi T. (2003) Diversity of the cadherin-related neuronal receptor/protocadherin family and possible DNA rearrangement in the brain. Genes Cells **8:** 1–8
35 Blaschuk O. W., Sullivan R., David S. and Pouliot Y. (1990) Identification of a cadherin cell adhesion recognition sequence. Dev. Biol. **139:** 227–229
36 Brieher W. M., Yap A. S. and Gumbiner B. M. (1996) Lateral dimerization is required for the homophilic binding activity of C-cadherin. J. Cell Biol. **135:** 487–496
37 Shan W. S., Koch A., Murray J., Colman D. R. and Shapiro L. (1999) The adhesive binding site of cadherins revisited. Biophys. Chem. **82:** 157–163
38 Takeda H., Shimoyama Y., Nagafuchi A. and Hirohashi S. (1999) E-cadherin functions as a cis-dimer at the cell-cell adhesive interface in vivo. Nat. Struct. Bio.l **6:** 310–312
39 Yap A. S., Brieher W. M., Pruschy M. and Gumbiner B. M. (1997) Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function. Curr. Biol. **7:** 308–315
40 Chappuis-Flament S., Wong E., Hicks L. D., Kay C. M. and Gumbiner B. M. (2001) Multiple cadherin extracellular repeats mediate homophilic binding and adhesion. J. Cell Biol. **154:** 231–243
41 Kitagawa M., Natori M., Murase S., Hirano S., Taketani S. and Suzuki S. T. (2000) Mutation analysis of cadherin-4 reveals amino acid residues of EC1 important for the structure and function. Biochem. Biophys. Res. Commun. **271:** 358–363
42 Shan W. S., Tanaka H., Phillips G. R., Arndt K., Yoshida M., Colman D. R. et al. (2000) Functional cis-heterodimers of N- and R-cadherins. J. Cell Biol. **148:** 579–590
43 Shimoyama Y., Tsujimoto G., Kitajima M. and Natori M. (2000) Identification of three human type-II classic cadherins and frequent heterophilic interactions between different subclasses of type-II classic cadherins. Biochem. J. **349:** 159–167
44 Tamura K., Shan W. S., Hendrickson W. A., Colman D. R. and Shapiro L. (1998) Structure-function analysis of cell adhesion by neural (N-) cadherin. Neuron **20:** 1153–1163
45 Ozawa M. (2002) Lateral dimerization of the E-cadherin extracellular domain is necessary but not sufficient for adhesive activity. J. Biol. Chem. **277:** 19600–19608
46 Troyanovsky R. B., Klingelhofer J. and Troyanovsky S. (1999) Removal of calcium ions triggers a novel type of intercadherin interaction. J. Cell Sci. **112:** 4379–4387
47 Chitaev N. A. and Troyanovsky S. M. (1998) Adhesive but not lateral E-cadherin complexes require calcium and catenins for their formation. J. Cell Biol. **142:** 837–846
48 Klingelhofer J., Laur O. Y., Troyanovsky R. B. and Troyanovsky S. M. (2002) Dynamic interplay between adhesive and lateral E-cadherin dimers. Mol. Cell. Biol. **22:** 7449–7458
49 Troyanovsky R. B., Sokolov E. and Troyanovsky S. M. (2003) Adhesive and lateral E-cadherin dimers are mediated by the same interface. Mol. Cell. Biol. **23:** 7965–7972
50 Pokutta S., Herrenknecht K., Kemler R. and Engel J. (1994) Conformational changes of the recombinant extracellular domain of E- cadherin upon calcium binding. Eur. J. Biochem. **223:** 1019–1026
51 Pertz O., Bozic D., Koch A. W., Fauser C., Brancaccio A. and Engel J. (1999) A new crystal structure, Ca2+ dependence and mutational analysis reveal molecular details of E-cadherin homoassociation. EMBO J. **18:** 1738–1747
52 Ahrens T., Pertz O., Haussinger D., Fauser C., Schulthess T. and 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
53 Ahrens T., Lambert M., Pertz O., Sasaki T., Schulthess T., Mege R. M. et al. (2003) Homoassociation of VE-cadherin follows a mechanism common to 'classical' cadherins. J. Mol. Biol. **325:** 733–742
54 Tomschy A., Fauser C., Landwehr R. and Engel J. (1996) Homophilic adhesion of E-cadherin occurs by a co-operative two-step interaction of N-terminal domains. EMBO J. **15:** 3507–3514
55 Sivasankar S., Gumbiner B. and Leckband D. (2001) Direct measurements of multiple adhesive alignments and unbinding trajectories between cadherin extracellular domains. Biophys. J. **80:** 1758–1768
56 Zhu B., Chappuis-Flament S., Wong E., Jensen I. E., Gumbiner B. M. and Leckband D. (2003) Functional analysis of the structural basis of homophilic cadherin adhesion. Biophys. J. **84:** 4033–4042
57 Sivasankar S., Brieher W., Lavrik N., Gumbiner B. and Leckband D. (1999) Direct molecular force measurements of multiple adhesive interactions between cadherin ectodomains. Proc. Natl. Acad. Sci. USA **96:** 11820–11824
58 Nagar B., Overduin M., Ikura M. and Rini J. M. (1996) Structural basis of calcium-induced E-cadherin rigidification and dimerization. Nature **380:** 360–364
59 Overduin M., Harvey T. S., Bagby S., Tong K. I., Yau P., Takeichi M. et al. (1995) Solution structure of the epithelial cadherin domain responsible for selective cell adhesion. Science **267:** 386–389
60 Overduin M., Tong K. I., Kay C. M. and 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
61 Shapiro L., Fannon A. M., Kwong P. D., Thompson A., Lehmann M. S., Grubel G. et al. (1995) Structural basis of cell-cell adhesion by cadherins. Nature **374:** 327–337
62 Shapiro L., Kwong P. D., Fannon A. M., Colman D. R. and Hendrickson W. A. (1995) Considerations on the folding topology and evolutionary origin of cadherin domains. Proc. Natl. Acad. Sci. USA **92:** 6793–6797
63 Boggon T. J., Murray J., Chappuis-Flament S., Wong E., Gumbiner B. M. and Shapiro L. (2002) C-cadherin ectodomain structure and implications for cell adhesion mechanisms. Science **296:** 1308–1313
64 Haussinger D., Ahrens T., Sass H. J., Pertz O., Engel J. and Grzesiek S. (2002) Calcium-dependent homoassociation of E-cadherin by NMR spectroscopy: changes in mobility, conformation and mapping of contact regions. J. Mol. Biol. **324:** 823–839
65 He W., Cowin P. and Stokes D. L. (2003) Untangling desmosomal knots with electron tomography. Science **302:** 109–113
66 Hyafil F., Babinet C. and Jacob F. (1981) Cell-cell interactions in early embryogenesis: a molecular approach to the role of calcium. Cell **26:** 447–454
67 Ozawa M., Engel J. and Kemler R. (1990) Single amino acid substitutions in one Ca^{2+} binding site of uvomorulin abolish the adhesive function. Cell **63:** 1033–1038
68 Tong K. I., Yau P., Overduin M., Bagby S., Porumb T., Takeichi M. et al. (1994) Purification and spectroscopic characterization of a recombinant amino-terminal polypeptide fragment of mouse epithelial cadherin. FEBS Lett. **352:** 318–322
69 Koch A. W., Pokutta S., Lustig A. and Engel J. (1997) Calcium binding and homoassociation of E-cadherin domains. Biochemistry **36:** 7697–7705
70 Katz B. Z., Levenberg S., Yamada K. M. and Geiger B. (1998) Modulation of cell-cell adherens junctions by surface clustering of the N-cadherin cytoplasmic tail. Exp. Cell Res. **243:** 415–424
71 Ozawa M. and Kemler R. (1998) The membrane-proximal region of the E-cadherin cytoplasmic domain prevents dimerization and negatively regulates adhesion activity. J. Cell Biol. **142:** 1605–1613
72 Tanaka H., Shan W., Phillips G. R., Arndt K., Bozdagi O., Shapiro L. et al. (2000) Molecular modification of N-cadherin in response to synaptic activity. Neuron **25:** 93–107
73 Laur O. Y., Klingelhofer J., Troyanovsky R. B. and Troyanovsky S. M. (2002) Both the dimerization and immunochemical properties of E-cadherin EC1 domain depend on Trp(156) residue. Arch. Biochem. Biophys. **400:** 141–147
74 Yap A. S., Niessen C. M. and Gumbiner B. M. (1998) The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening and interaction with p120ctn. J. Cell Biol. **141:** 779–789
75 Huber O., Kemler R. and Langosch D. (1999) Mutations affecting transmembrane segment interactions impair adhesiveness of E-cadherin. J. Cell Sci. **112 (Pt 23):** 4415–4423
76 Lambert M., Padilla F. and Mege R. M. (2000) Immobilized dimers of N-cadherin-Fc chimera mimic cadherin-mediated cell contact formation: contribution of both outside-in and inside-out signals. J. Cell Sci. **113 (Pt 12):** 2207–2219
77 Alattia J. R., Ames J. B., Porumb T., Tong K. I., Heng Y. M., Ottensmeyer P. et al. (1997) Lateral self-assembly of E-cadherin directed by cooperative calcium binding. FEBS Lett. **417:** 405–408
78 Baumgartner W., Hinterdorfer P., Ness W., Raab A., Vestweber D., Schindler H. et al. (2000) Cadherin interaction probed by atomic force microscopy. Proc. Natl. Acad. Sci. USA **97:** 4005–4010
79 Wahl J. K. 3rd, Kim Y. J., Cullen J. M., Johnson K. R. and Wheelock M. J. (2003) N-cadherin-catenin complexes form prior to cleavage of the proregion and transport to the plasma membrane. J. Biol. Chem. **278:** 17269–17276
80 Niessen C. M. and Gumbiner B. M. (2002) Cadherin-mediated cell sorting not determined by binding or adhesion specificity. J. Cell Biol. **156:** 389–399
81 Karecla P. I., Green S. J., Bowden S. J., Coadwell J. and Kilshaw P. J. (1996) Identification of a binding site for integrin alphaE-beta7 in the N-terminal domain of E-cadherin. J. Biol. Chem. **271:** 30909–30915
82 Whittard J. D., Craig S. E., Mould A. P., Koch A., Pertz O., Engel J. et al. (2002) E-cadherin is a ligand for integrin alpha2beta1. Matrix Biol. **21:** 525
83 Ozawa M. and Kemler R. (1990) Correct proteolytic cleavage is required for the cell adhesive function of uvomorulin. J. Cell Biol. **111:** 1645–1650
84 Leckband D. and Sivasankar S. (2000) Mechanism of homophilic cadherin adhesion. Curr. Opin. Cell Biol. **12:** 587–592
85 Nagafuchi A. (2001) Molecular architecture of adherens junctions. Curr. Opin. Cell Biol. **13:** 600–603


To access this journal online:
http://www.birkhauser.ch