CMLS, Cell. Mol. Life Sci. 55 (1999) 359–367
1420-682X/99/030359-09 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Structural view of cadherin-mediated cell-cell adhesion

J. R. Alattia, H. Kurokawa and M. Ikura*

Division of Molecular and Structural Biology, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto (Ontario M5G 2M9, Canada), Fax + 1 416 946 6529, e-mail: mikura@oci.utoronto.ca

Received 14 September 1998; received after revision 14 November 1998; accepted 16 November 1998

Abstract. Following the multiplication of biochemical, biophysical and structural studies describing cadherin molecules and their interactions, several ideas have emerged to explain the mechanisms of cadherin-mediated cell adhesion. Although different models were proposed for cadherin interactions, a consensus has come forth considering lateral dimerization of cadherins as being a central component of the cell-cell adhesion process. This review summarizes the recent development in structural studies of cadherins.

Key words. Cell adhesion; cadherin; calcium; dimerization; protein interaction.

Introduction

The wide majority of cells in multicellular organisms are arranged into cohesive assemblies, and among these many can interact preferentially with cells of their own type. This homophilic cell adhesion that allows sorting of cells during morphogenesis is mediated by surface and transmembrane proteins. It has long been known that calcium is a key element in maintaining cellular adhesiveness, as shown from live tissues and cultured cells dissociating whenever exposed to a calcium-depleted environment [1, 2]. Cadherins represent a major superfamily of transmembrane glycoproteins that mediate calcium-dependent cell-cell adhesion in both vertebrates and invertebrates [3, 4]. As summarized in table 1, the cadherin superfamily is divided into several families according to the number of repeats in the extracellular domain [5, 6]. Typically composed of five extracellular repeats [7], the family of classic cadherins is the best characterized, and known to serve cell-cell adhesion functions. Others, including desmosomal cadherins [8–10] and the protooncogene product RET (the acronym stands for 'rearranged during transfection') [11–13], may function as cell adhesion molecules, but could also serve different biological roles.

In recent years, structural data were acquired through studies using both X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, which tremendously contributed to our understanding of the anatomy of cadherin molecules and their functionality, but several questions remain unanswered and new ones are raised, including the intimate mechanism of cell-cell adhesion. We will concentrate in this review on the structural aspects of classic cadherins, and expose the different models suggested for the adhesion process.

Organization of the cadherin molecule

In general, cadherin molecules possess functional sites for adhesive recognition, calcium binding, membrane integration, cytoskeletal interactions and posttranslational modifications such as glycosylation, phosphorylation and proteolysis [5]. Classic cadherins are transmembrane proteins with an extracellular domain located in the N-terminal part of the molecule. The

* Corresponding author.

Table 1. The cadherin superfamily.

Five repeats			More than five repeats	Unrelated proteins
Classic cadherins	cadherins with no cytoplasmic domain	desmosomal cadherins		
Group 1*				
E-cadherin				
N-cadherin				
R-cadherin				
P-cadherin	T-Cadherin	Desmoglein 1–3		
B-cadherin		Desmocollin 1–3	Fat (34 repeats)	c-Ret
L-CAM			PC42 (7 repeats)	
EP-cadherin			PC43 (6 repeats)	
U-cadherin				
XB-cadherin				
DE-cadherin‡†				
Group 2§				
Cadherin 6, 8–11				
Others				
M-cadherin				
Cadherin 5				

*Mainly localized in adhaerens junctions. †Actually has six repeats, but the first repeat seems to be cleaved upon maturation [37]. ‡DE-cadherin is functionally similar to vertebrate classic cadherins. §Localization and function not fully identified.

intracellular domain is linked to actin filaments via α- and β-catenin and is known to be involved in the Wnt/APC (Wnt is a growth factor; APC is a protein named for adenomatosis polyposis coli) signaling pathway [14–16]. The extracellular domain is typically made of five repeats, referred to as 'domains' in what follows, each about 110 amino acids long (fig. 1).

A peptide sequence (His-Ala-Val) was identified in several N-terminal domains of classic cadherins and is thought to play a role in their homotypic binding [17, 18]. Residues flanking this His-Ala-Val sequence are thought to contribute to the binding specificity. Other parts of the molecule are probably involved in the mechanism underlying cell-cell interactions as evidenced by several experimental facts (reviewed by Humphries and Newham [7]). First, adhesion-blocking monoclonal antibodies are directed against the membrane-proximal region; second, mutations close to the transmembrane region reduce adhesion; third, mutations in the calcium binding sites outside the N-terminal repeat can be inhibitory. It is therefore likely that lateral interaction of cadherins, as in certain immunoglobulin-like adhesion proteins, is a prerequisite for stable cell-cell adhesion [9, 19, 20].

The cadherin fold

The structures of four different cadherin domains have been determined and were shown to share the same features [21–24]. The fold consists of a seven-strand β-sheet (A, A′, B, C, D, E, F and G. A and A′ are parts of the same strand), with the N and C termini located at opposite ends of the molecule. Its sandwich structure is similar to that of variable and constant domains of the immunoglobulin (Ig) superfamily [25], although there are no sequence similarities. However, it differs by resembling more a barrel than a sandwich and exhibiting two small helices in place of the disordered B–C and E–F loops (figs 2, 3). Indeed, the segment connecting strands B and C adopts an apparently helical structure made of a succession of β-turns and β-like hydrogen bonds. This unique quasi-β-helix structure is characteristic of the cadherin fold. In addition, the conserved disulfide bond between strands B and F in the Ig fold is absent in cadherin domains. Domain 1 of N-cadherin (Ncad1) and domain 2 of E-cadherin (Ecad2) have a small A strand. Two sets of strands form two twisted β-sheets: one with strands A', G, F and C, and the other with strands A, B, E and D.

Ncad1 and domain 1 of E-cadherin (Ecad1) have a similar fold, but they differ slightly by four residues at the N terminus and small displacements in loops B-C and F-G. In Ncad1, β-strands are exchanged between adjacent molecules and will be discussed below in terms of dimer formation. The sequence of Ncad1 has 57% residue identity with Ecad1. In comparison, Ecad1 and Ecad2 share only 25% identity, and accordingly there are more structural differences between them, mainly in loop regions [26].

Calcium binding

The crystal structure of the E-cadherin fragment containing domains 1 and 2 (Ecad12) showed that calcium is central in E-cadherin dimer formation. The residues that are involved in calcium binding are located in the linker region or its vicinity, at the interface between domains 1 and 2. Two types of interactions between the calcium binding sites were observed [23]. First, intramolecular interactions involving residues Glu11, Glu69, Asp103 and Asp136 link the three calcium ions bound to the Ecad12 monomer (fig. 4). Second, residues Asp100, Gln101, Asn102 and Asn143 coordinate calcium ions on one monomer while forming intermolecular hydrogen bonds with the opposite monomer, either directly or via water molecules (fig. 5). The orientation of the cadherin molecules would therefore be fixed by the extensive network of interacting Ca^{2+} ions, water molecules and Ecad12 side chains. Sequence similarity between the different domains (fig. 5) suggests that similar calcium binding sites might be found between other pairs of domains.

Calcium binding by Ecad12 is highly cooperative, as demonstrated by flow dialysis experiments [19]. The apparent calcium dissociation constant measured at different protein concentrations is 23 μM suggesting that the affinity to calcium is similar between monomeric and dimeric cadherin. This dissociation constant is also similar to the average value determined for the entire extracellular region of E-cadherin (30 μM) [27], indicating that the Ecad12 fragment which spans the two N-terminal domains of E-cadherin retains the calcium-binding properties of the whole extracellular region.

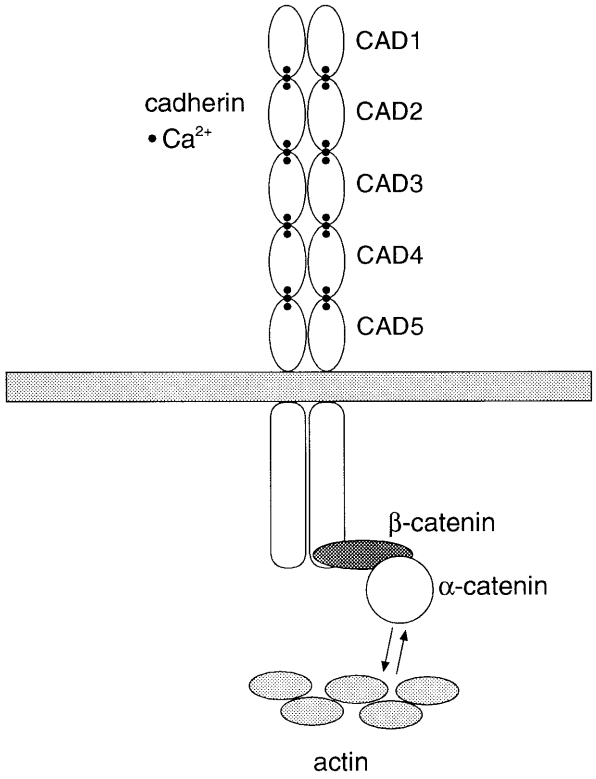

Figure 1. Schematic representation of classic cadherin molecules at the cell surface.

Dimer formation

Crystal structures of both Ecad12 and Ncad1 show parallel dimer formation, although the modes of interaction are different. In Ecad12 [23] the contacts are mostly located in the linker region between the domains and are mediated by calcium ions and water molecules (fig. 6B). Residues that coordinate calcium ions on one molecule form mainly water-mediated hydrogen bonds with residues from the opposite molecule. Gln101 is the only calcium-chelating residue to form direct hydrogen bonds. Domains 1 and 2 do not interact extensively, burying in total only 450 $Å^2$ [23]. The total surface area buried in the dimer interface is 2000 $Å^2$ of which 1000 $Å^2$ are accounted for by residues in the N-terminal domain. In contrast, residues from domain 1 in Ncad, described below, bury 1800 $Å^2$ in the strand dimer interface.

In the crystal structure of the N-cadherin single domain [22], the N-terminal A strand is exchanged between two interacting molecules to form an intimate 'strand' dimer. The most striking feature of this homodimer is the intercalation of the Trp 2 side chain into the hydrophobic core of the partner (fig. 6A). In support of this model, it was found that point mutations of residues in the vicinity of the crossing-over A strands in the N-terminus repeat had no effect on cadherin expression or presentation at the cell surface, but cadherin-mediated cell adhesion was often completely abolished [24]. Tamura et al. [24] mutated Trp 2 in E-cadherin, which resulted in a total loss of cell-cell adhesion. Unfortunately, they did not perform the same mutation in N-cadherin. The authors interpreted the results in terms of the strand dimer model, but they could not dismiss other possible mechanisms.

This type of interface was not observed in a subsequent crystal structure of a dual N-cadherin construct containing the two N-terminal domains (Ncad12) [24]. Remarkably, the N-terminal tip, including the large hydrophobic Trp 2 residues, was disordered in this structure. In the solution structure of Ecad1 [21] and the crystal structure of Ecad12 [23], the N-terminal tip was similarly disordered, and it was claimed that the disorder was due to additional residues at the N-termi-

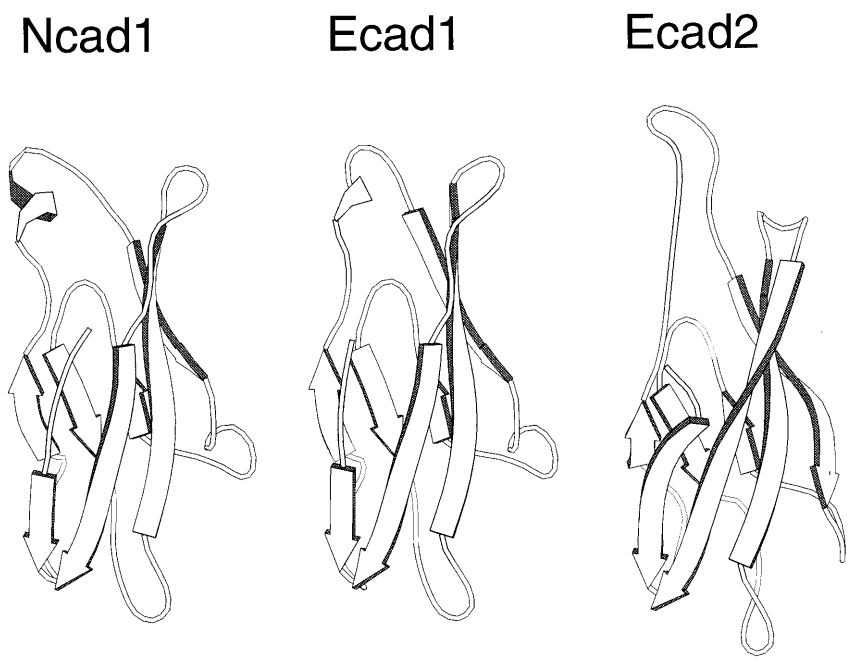

Figure 2. Different folds of cadherin repeats. Ncad1 and Ecad1 share 57% sequence identity and have very similar folds. There are more structural differences between Ecad1 and Ecad2, which share only 25% sequence identity.

nus (Met-Arg) remaining from the cloning procedure. However, the results obtained by Tamura et al. [24] demonstrate that cadherin molecules with no extra residues can be similarly disordered.

There is increasing evidence in favor of Ca^{2+}-mediated dimer formation in cadherins. In the case of Ecad12, sedimentation equilibrium experiments have shown that Ecad12 is monomeric in the absence of calcium. When calcium is present, a monomer-dimer equilibrium of Ecad12 is established; the calculated dissociation constant for the dimerization is 0.17 mM [19]. Calcium binding is highly cooperative at protein concentrations allowing dimer formation. The cooperativity decreases at low protein concentrations where Ecad12 is monomeric. This is in favor of a system where calcium ions are implicated in dimer formation. Electron microscopy images of Ca^{2+}-free Ecad12 showed single molecules, each of which contains two domains with variable relative orientations, suggesting a flexible interdomain linker as previously demonstrated by Pokutta et al. for the entire extracellular domain [28]. On the other hand, images of Ca^{2+}-bound Ecad12 showed four domains closely disposed as one would expect to see in a dimer [19].

It is unlikely that the two types of dimer interfaces can be accommodated in a single model, since dimerization by calcium coordination precludes Trp 2-dependent association. This led to the suggestion that N- and E-cadherin might use different molecular mechanisms. Despite the high degree of sequence homology between the two cadherins, differences can be found at critical points. For example, Gln101, which is involved in direct and water-mediated hydrogen bonding between Ecad12 monomers, is replaced in Ncad12 by a methionine [24]. However, the residues that coordinate calcium ions are strictly conserved between the two cadherins, although they do not form a dimer interface in the crystal structure of Ncad12. The explanation for that may lie in the fact that cadherin dimerization is weak, as shown by sedimentation equilibrium data for Ecad12 [19], so that the lack of an element from the interface, namely the hydrogen bonding Gln101, weakens an already weak association and eventually abolishes it, especially if constraining crystal packing forces are involved. It is interesting to note that the crystallization conditions were different between E-cadherin and N-cadherin fragments. Crystals of Ecad12, Ncad1 and Ncad12 grew, respectively, in 10 mM calcium chloride at pH 9.0, pH 5.0 with no calcium and pH 5.0 with 15 mM calcium chloride. The difference in pH of the crystallization buffer might affect crystal packing.

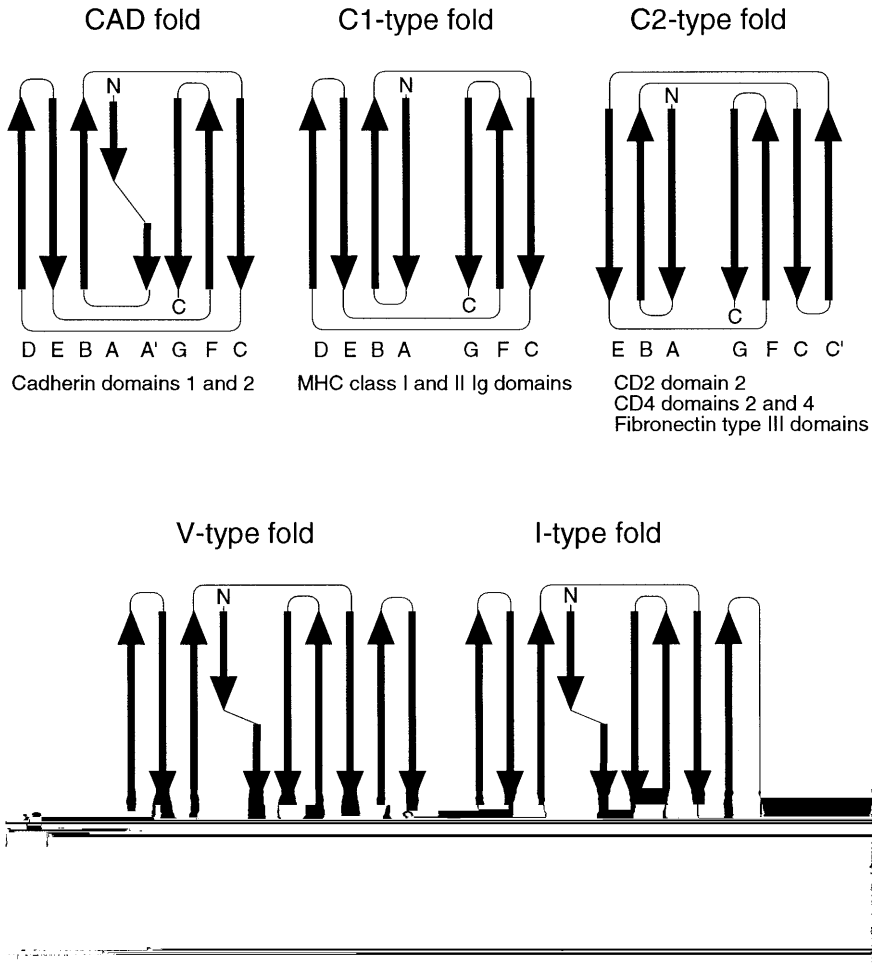

Figure 3. Schematic drawings comparing the topologies of cadherin and different folds from the immunoglobulin superfamily (IgSF).

Higher organization of cadherin molecules and cell adhesion

Mounting evidence seems to support that dimerization of cadherin molecules is a prerequisite for cell-cell adhesion. We have shown [19] that dimer formation is weak in the case of Ecad12. In vivo studies [29] have directly demonstrated the fundamental role of lateral clustering in cadherin function. The distribution of cadherin molecules presented at the cell surface, possibly regulated by the cytoplasmic domain and its connections, can modulate cellular adhesion. Other biochemical data [20] have even suggested that there are two states of cadherin-mediated adhesion. The first, relatively weak, is mediated by the extracellular segment only. The second, strong, requires the cytoplasmic tail and possibly its linkage to the cytoskeleton. In any case, grouping, as well as organizing cadherin structures, can yield the necessary strength for adhesion. Although electron microscopy imaging of extracellular cadherin constructs suggested that interactions between cadherin monomers are limited to the N-terminal tip of the molecule [30], there is increasing evidence that other extracellular parts of cadherins and/or the intracellular domain are involved in lateral clustering and ultimately cell adhesion [19].

The role of calcium in cell-cell interactions

As mentioned earlier, calcium ions are necessary for the adhesive activity. Single amino acid substitutions in the calcium binding sites can disrupt cell aggregation in vivo [31]. Early biochemical and biophysical data suggested several explanations for this dependence, including rigidifying cadherin structure [28] and conferring resistance to proteolysis [32].

How does calcium play its role in cell-cell interactions? Brieher et al. [20] reported that dimerization of cadherin molecules could occur regardless of the presence or absence of Ca^{2+} ions, whereas adhesive head-to-head binding interactions might be strictly calcium-dependent (fig. 7A). This model is supported by in vitro aggregation experiments, and biochemical cross-linking of the extracellular domain of C-cadherin [20]. The fact that it involves calcium-independent dimerization is consistent with the 'strand dimer' of N-cadherin reported by Shapiro et al. [22]. Recently reported in vivo data reached the same conclusion [33] and added that both catenins and calcium are essential for adhesive but not for lateral interactions. This conclusion disagrees in part with another recent report by Ozawa and Kemler [34] that showed that E-cadherin lacking the entire cytoplasmic domain mediates Ca^{2+}-dependent cell aggregation to the same extent as wild-type E-cadherin. These results suggest that the cytoplasmic domain contains a membrane-proximal region that prevents dimerization and cell adhesion, whereas the distal region promotes the opposite effect through its interaction with catenins. Despite several unanswered questions and apparent contradictions, all in vitro and in vivo studies seem to underline the importance of cadherin dimerization in cell adhesion.

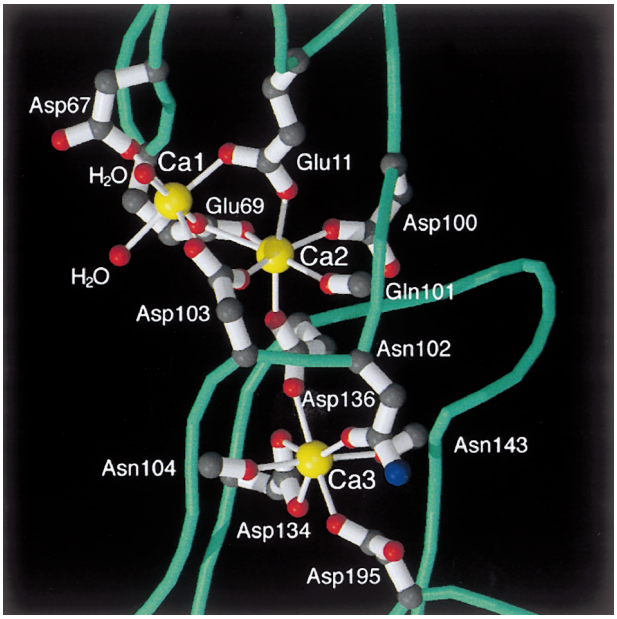

Figure 4. Calcium binding sites on single Ecad12 molecules. Glu11 and Glu69 bridge Ca1 and Ca2, whereas Asp136 bridges Ca2 and Ca3. Two water molecules are involved in the coordination of Ca1.

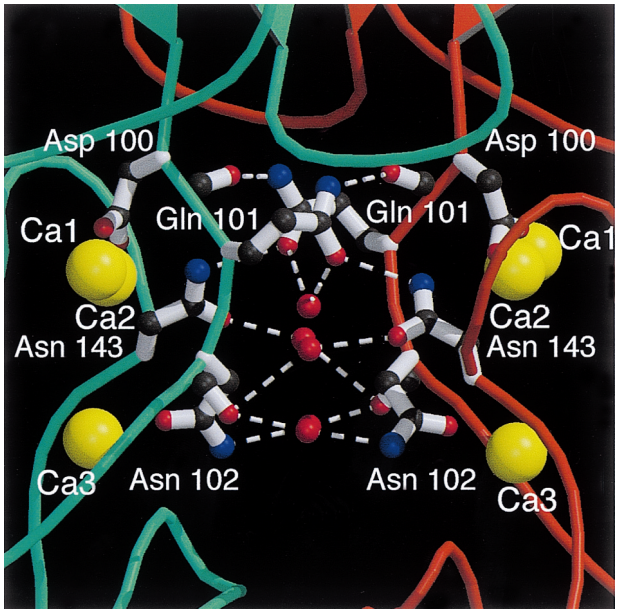

Figure 5. Atomic detail of the calcium-mediated interface in the Ecad12 dimer. Gln101, which is absent in N-cadherin molecules, coordinates ion Ca^{2} on one molecule while forming hydrogen bonds with the opposite molecule. Water molecules are also involved in hydrogen bonds bridging the two Ecad12 monomers.

In contrast, our structural [23] and biophysical studies [19] on E-cadherin suggest a model (fig. 7B) in which Ca^{2+} ions directly mediate dimer formation, but adhesive contacts are the result of direct protein-protein interactions. In this model, Ca^{2+} ions are responsible for securing the proper geometry for adhesive contacts (fig. 8) [19]. First, apo-cadherin monomers with flexible linkers bind Ca^{2+} ions, resulting in the overall rigidification of the molecule. Similarly important is that calcium binding restrains the positions of the individual adhesion sites to those suitable for the formation of a uniform cell-cell adhesion lattice. Ca^{2+} ions, and metal ions in general, can potentially provide the geometrical scaffolding through their coordinating valences, allowing a highly accurate positioning of the polypeptide chain with almost a 1-Å precision. Such organized structures at low concentrations would associate loosely, as exemplified by the weakness of the Ecad12 dimer in solution. However, the key to stable adhesion would lie in the collective assembly of a number of properly oriented structures. If a critical concentration of cadherin molecules is met at the cell surface, dimer formation occurs with a geometry guided by Ca^{2+} ions. The dimerization step, together with possible grouping events originating in the cytoplasm or involving other cadherin repeats, are crucial for the formation of an organized assembly.

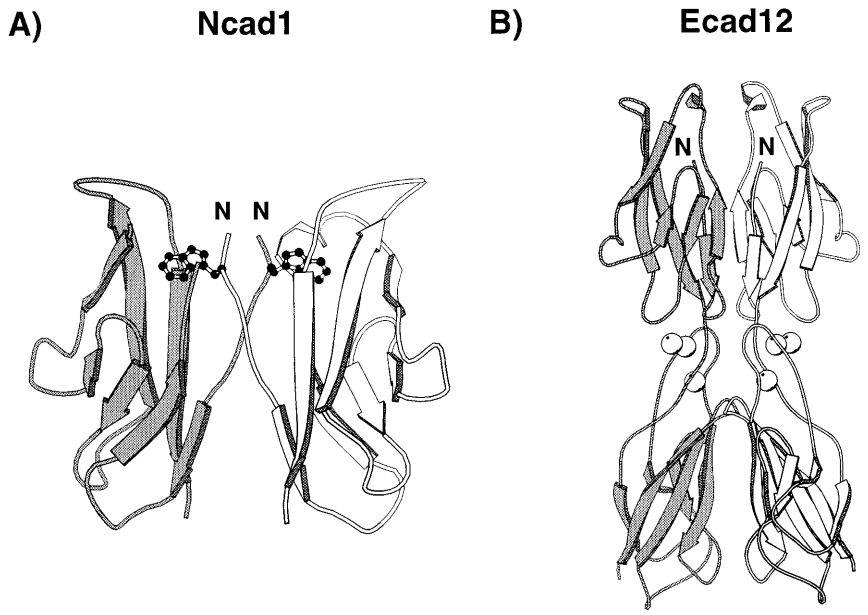

Figure 6. Comparison of dimer formation modes between N-cadherin and E-cadherin. (*A*) Trp2-mediated 'strand dimer' interface of Ncad1 molecules. (*B*) calcium-mediated dimer interface between Ecad12 molecules. The structure shown in (*A*) is enlarged by a factor of 1.5 with respect to the structure in (*B*).

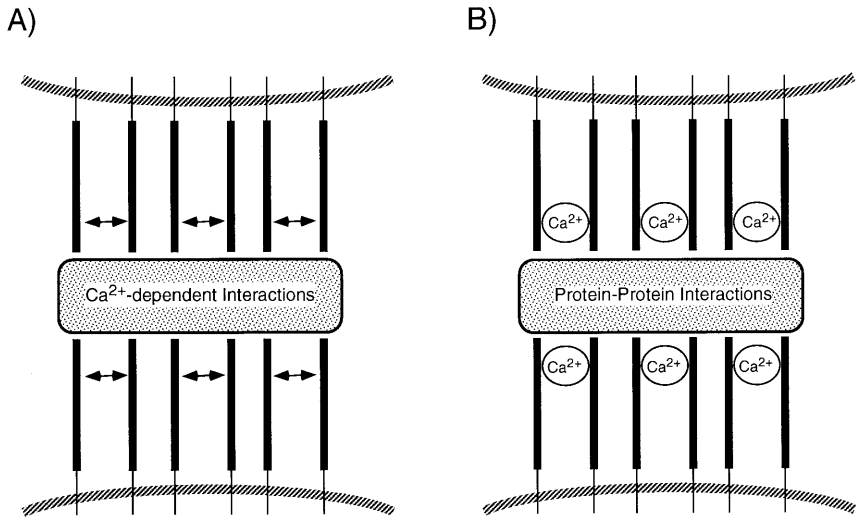

Figure 7. Schematic drawing representing different models for lateral dimerization and adhesion. Extracellular domains of cadherin molecules are represented by thick vertical bars. (*A*) Lateral dimerization of extracellular cadherin domains is Ca^{2+}-independent, whereas adhesion interactions are Ca^{2+}-dependent. (*B*) Calcium is directly involved in lateral dimer formation, but adhesive contacts are secured by protein-protein interactions.

Finally, the cadherin cluster can interact with a geometrically compatible assembly that is similarly formed at the opposite cell surface to achieve cadherin-mediated cell-cell adhesion.

Towards unraveling the mechanism of cell-cell adhesion

The latest findings indicate that multimolecular grouping events are necessary for cadherin to achieve cell

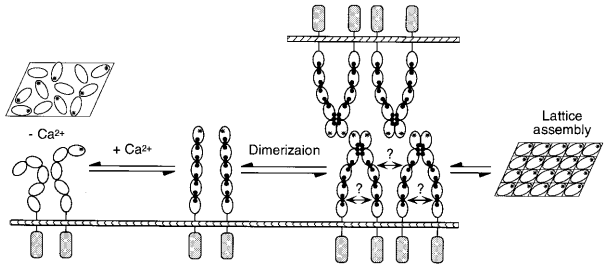

Figure 8. Schematic drawing of a proposed mechanism for calcium-mediated assembly of cadherin molecules. Extracellular cadherin repeats are represented by open ovals, Ca^{2+} ions by small dark spheres, adhesion sites by asterisks and the cytoplasmic domains by shaded rectangles. The disordered ($-Ca^{2+}$) cadherin lattice is represented at the top, where adhesion sites might be unformed or assume random orientations.

adhesion, possibly involving other repeats in the molecule [19, 20]. Having an integrated view of the entire extracellular portion of cadherin, the one directly involved in the cell-cell interface, turns up to be more crucial than ever. In order to attain it, a working strategy may involve the characterization of the remaining polypeptide segments that are amenable to the major structural methods, namely NMR and X-ray crystallography.

The cadherin extracellular domain contains five cadherin repeats that have probably evolved from a single ancestral module. On the basis of structure-based alignments [21, 35], sequence identities are found to be greatest among repeats 1–4 (19–29% for E-cadherin), whereas the fifth domain appears less related to the others (10–16%), although it retains notable features. The first gene duplication would have produced a dual cadherin domain, a minimal structural entity as demonstrated by the crystal structure of Ecad12 [23] where both repeats and the linker bind the critical calcium ions. The second duplication would have produced a double dual domain which has a total of four similar repeats. The fifth repeat was probably added later or diverged earlier for undetermined reasons. If this hypothesis is correct, the E-cadherin fragment made of repeats 3 and 4 would be similar to the N-terminal fragment, Ecad12, which has already been elucidated by X-ray crystallography [23]. The key to the reconstruction of the entire extracellular portion would lie in the knowledge of how these two fragments are spatially disposed in relation to each other. Future structural work will be focused on elucidating the entire extracellular domain, as well as the more elusive intracellular segment interacting with β-catenins. The structure of the β-catenin *armadillo* repeat was recently reported by Huber et al. [36] and will ultimately serve, in conjunction with the information available for cadherins, as a basis for understanding the molecular cross-talk between cell-cell adhesion and various signaling and cell development events.

Acknowledgments. We thank Dr. Michael Overduin for his help in preparing figure 3. This work was supported by grants to M.I. from the National Cancer Institute of Canada. J.-R. A. acknowledges the Caven postdoctoral fellowship. M.I. is a Howard Hughes Medical Research International Research Scholar.

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