Cadherin structure: a revealing zipper

The crystal structure of the N-terminal domain of neural cadherin provides the first atomic-level picture of interacting cell-adhesion molecules, and suggests a mechanism for assembly and disassembly of intercellular adhesion zones.

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The development and maintenance of tissues depends critically upon cells of the same type adhering to one another. The cadherin cell-adhesion molecules mediate Ca^{2+}-dependent, homophilic interactions: cells expressing the same type of cadherin adhere to one another, and mixtures of cells expressing different cadherins segregate according to cadherin type [1]. This function is crucial to the sorting of cell types into defined tissue structures during development. In mature tissues, cadherins play a part in maintaining differentiated cell structure. For example, expression of epithelial (cadherin in mesenchymal cells that normally display a uniform Na^+/K^+ ATPase distribution is sufficient to induce redistribution of the enzyme to sites of cell–cell contact similar to that found in polarized epithelia [2]. Cadherins also have roles in determining the abnormal adhesive properties of transformed cells. Certain non-invasive epithelial cells acquire invasive properties characteristic of metastasizing cells when cadherin function is disrupted [3,4], and transfection of invasive epithelial tumor cell lines with E-cadherin cDNA suppresses invasiveness [4].

Members of the cadherin superfamily are found in both vertebrates and invertebrates, and include the classical vertebrate cadherins found in cell–cell adherens junctions, the desmogleins and desmocollins of desmosomes, and others of less certain cellular localization. All are type I membrane proteins whose extracellular portion contains variable numbers of a characteristic ~110 amino acid repeat referred to here as a cadherin domain (CAD). The best-studied cadherins are the classical cadherins, which contain five CADs. Various lines of evidence have implicated the N-terminal domain (CAD1) in determining homophilic binding specificity [5,6]. Recently, structures of CAD1 from two of the classical cadherins, neural (N) and epithelial (E), have been determined by crystallographic (N-CAD1) [7] and solution NMR (E-CAD1) [8] methods. The structures provide a framework for understanding the conserved repeat sequence and Ca^{2+} requirement of cadherins. Moreover, the organization of N-CAD1 in several different crystal lattices may reflect the interactions of cadherins at cellular junctions and suggests how these interactions contribute to the dynamics of cell–cell adhesion.

Structure of the cadherin domain and Ca^{2+} binding

The cadherin domain is approximately 45 Å×25 Å×25 Å and consists of seven antiparallel β-strands (designated A to G) arranged in a 'Greek key' topology similar to that of the immunoglobulin (Ig) fold, but with a series of β-turns resembling a helix found between strands C and D (the 'quasi β-helix' [7]). Structure-based sequence alignments indicate that the other four domains in the extracellular portion of classical cadherins, as well as other cadherin domains, adopt a fold similar to that of CAD1. The similarities of the cadherin and Ig folds raise the possibility that these two families of adhesion molecules are derived from a common ancestor [9]. However, differences in gene structure, distinct hydrophobic cores, and the presence of this fold in a diverse set of proteins, tend to favor the notion that cadherins and Ig superfamily members evolved independently [10].

The N and C termini are located at opposite ends of the cadherin domain, such that tandemly repeated domains can form an elongated molecule. In both N-CAD1 and E-CAD1, the Ca^{2+}-binding residues lie at the same end of the domain as the C terminus (Fig. 1), and form an incomplete set of coordination ligands for the divalent cation. Sequence comparisons reveal several acidic ligands at the N-terminal end of CADs 2–5, including a conserved aspartate in CAD2 previously implicated in

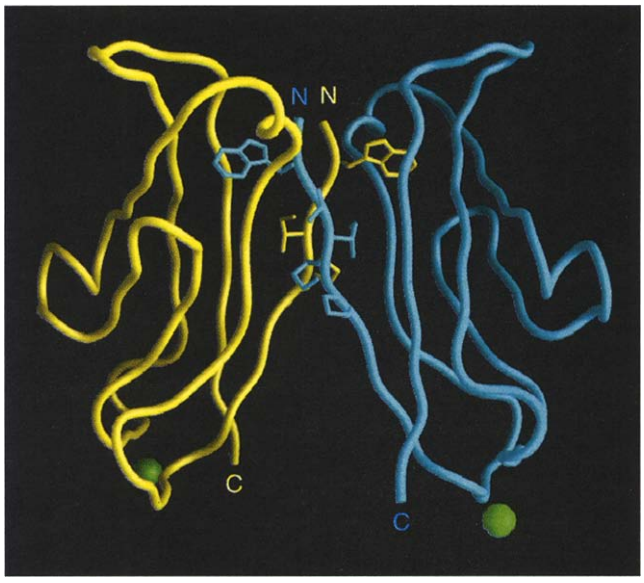

Fig. 1. Structure of two protomers interacting in the strand dimer. The N and C termini are labeled. Note that Trp2 is buried in the core of the partner molecule. The Ca^{2+}-binding sites are marked by green spheres. (Reproduced from [7], with permission.)

Ca^{2+}-binding [11], whereas CAD5 lacks the acidic residues equivalent to those in CAD1 that ligate Ca^{2+}. These observations suggest that the acidic residues seen at the C-terminal end of one domain, along with the acidic residues at the N-terminal end of the successive domain, form a Ca^{2+}-binding site that stabilizes the interface between successive domains to give a stiff, elongated molecule [7,8] (Fig. 2). Indeed, rod-like structures appear in electron micrographs of the E-cadherin extracellular region in the presence of Ca^{2+}, whereas more globular images are obtained when Ca^{2+} is removed [12]. The Ca^{2+} dependence of cadherin function therefore appears to be due, at least in part, to the need to maintain the molecule in a properly oriented, rigid state, rather than to a direct participation of Ca^{2+} in the adhesive interface. Nonetheless, addition of Ca^{2+} to E-CAD1 induces changes in the chemical shifts of residues His79 and Met92, found in dimer interfaces in the crystal structure (see below), suggesting that Ca^{2+} may affect residues involved in adhesive contacts.

Oligomeric structure and homophilic binding specificity

N-CAD1 is a dimer in solution [7], whereas E-CAD1 [13] and the entire extracellular portion of E-cadherin [12] appear to be monomeric. In both cases, these observations are independent of Ca^{2+}. The reason for this discrepancy is unclear, although the methods used to assess oligomeric state were different in the two cases. In each of the three crystal forms of N-CAD1, two kinds of dimer are observed. In the so-called 'strand' dimer [7], protomers associate in a parallel, side-by-side manner that features pairing of the beginning of strand A with strand B of the partner molecule and burial of Trp2 in the hydrophobic core of the partner molecule (Fig. 1). (In the E-CAD1 structure, residues 1–3 are solvent exposed; the backbone at positions 4 and 5 pairs with strand B of the same protomer.) A conserved phenylalanine in CADs 2–4 at the position equivalent to Trp2 suggests that these domains may also associate in this manner [7]. The other dimer forms a head-to-head complex in which residues from the quasi β-helix, the C strand, and the FG strand of one protomer interact with the quasi β-helix, C strand, and DE strands of the partner protomer, respectively. The fact that the same dimers are found in independent crystal forms of N-CAD1 strongly suggests a functional role, with the organization in the crystal lattices mimicking the arrangement at the cell surface [7].

As the strand dimer consists of parallel domains that would correspond to dimers projecting from the same cell surface, the head-to-head dimer seems likely to represent the interaction of cadherins from opposing cells, and has been termed the 'adhesion' dimer [7]. Although the few positions known to be determinants of homophilic binding specificity map to the adhesion dimer interface [5,6], the relevance of the observed interactions remains to be tested experimentally. Significantly, the character of residues observed to interact in the adhesion dimer interface varies in tandem among the cadherins.

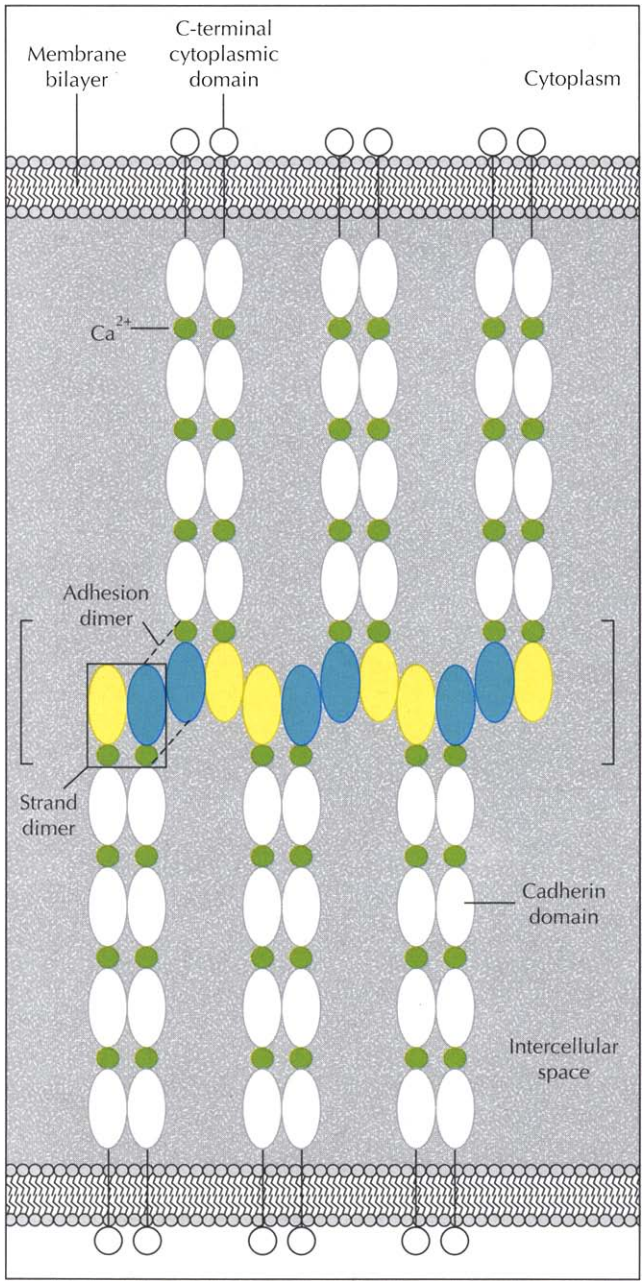

Fig. 2. Schematic diagram of an adherens junction. The cadherin protomer is shown with its five cadherin domains linked rigidly by Ca^{2+} (see text). Protomers are assumed to associate through their N-terminal domains as seen in the crystal structure of N-CAD1 [7]. The repeating array seen in the crystal lattices is bracketed. The cadherin domains are shown as ovals, with Ca^{2+} as green circles. The strand dimer (boxed) is shown with the same color scheme as Figure 1; the adhesion dimer is also outlined. In the model presented in [7], the opposing cell membranes are separated by 290 Å, consistent with electron-microscopic observations of the adherens junction.

For example, in N-cadherin, Ile56 of the DE loop and Ile83 in the FG loop form a hydrophobic contact; in E-cadherin these residues are replaced by glutamate and serine, respectively. In the one known case of cadherin-mediated heterophilic binding, that of N- and retinal (R)-cadherin, residues from N-cadherin and R-cadherin in these interfaces are for the most part identical, and all

are conserved in character. Thus, different specificities may arise from co-variation of interface residues that preserves complementary character [7].

Structure and dynamics of adhesion sites

Central to the function of cadherins is the linkage of their cytoplasmic domains to the cytoskeleton by intracellular attachment proteins. Cadherins lacking a cytoplasmic domain bind to soluble cadherins but do not support cell adhesion (reviewed in [14]). Engagement of the extracellular region of cadherins triggers cytoskeletal reorganization; conversely, disruption of the cytoplasmic cadherin–catenin complex leads to loss of adhesion. The coupling of cadherin binding and cytoskeletal reorganization is believed to be responsible for changes in cell morphology during development of solid tissues, as well as for maintaining the structure of the differentiated cell. Classical cadherins in cell–cell adherens junctions are linked to the actin cytoskeleton by α-catenin in conjunction with either β-catenin or plakoglobin [14].

In the N-CAD1 crystal lattices, the strand and adhesion dimers form an infinite repeat in which strand dimers interlock like teeth in a zipper [7] (Fig. 2). Although the association constant for interacting cadherin monomers is not known, it is presumably weak, given that in solution either monomers or dimers are seen. The zipper structure can be viewed as an oligomeric interaction at the cell surface in which many weakly interacting cadherins form a strongly adherent junction [7]. Shifting the equilibrium between strand dimers and higher-order multimers would correspond to zippering or unzippering the junction.

How might the zipper help us to understand the coupling of cadherin binding and cytoskeletal changes? The rigid, interlocked extracellular structure would produce a linearly arrayed cadherin–catenin complex in the cytoplasm. Such an array could serve as an organizing center for assembly of the filament bundles that run parallel to the plane of the membrane in adherens junctions and desmosomes [7]. Assembly of the cytoplasmic junction would stabilize the multimeric association of cadherins and insure a strongly adhesive patch at the cell surface. On the other hand, it is unlikely that the cytoplasmic filament system directly potentiates cell adhesion by positioning the extracellular zipper structure [7], because there must be a mechanism that localizes filaments to sites of cell–cell contact. Rather, the catenins probably regulate adhesion from the cytoplasmic side of the junction, as they are the targets of signals that affect cell growth and adhesiveness [14]. Signals that promote disassembly of catenins from the junctional complex probably weaken adhesion through dissociation from the cytoplasmic filament system (i.e. reversing the assembly process outlined above). There may also be more direct effects of catenins on cadherin multimerization; for example, α-catenin contains a domain homologous to the self-association domain of vinculin, another intracellular attachment protein [15,16]. Thus, even in the absence of cytoskeletal components catenins may affect the oligomeric state of cadherins.

Although these concepts need to be tested experimentally, the arrangement of molecules in the crystals of N-CAD1 provides a compelling picture of cell-surface cadherin interactions and a structural basis for the dynamics of cadherin-mediated cell–cell adhesion. It is somewhat ironic that a zippered-up crystal has given us such a good peek at a cell–cell junction.

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