Review

Thinking outside the cell: how cadherins drive adhesion

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Cadherins are a superfamily of cell surface glycoproteins whose ectodomains contain multiple repeats of β-sandwich extracellular cadherin (EC) domains that adopt a similar fold to immunoglobulin domains. The best characterized cadherins are the vertebrate 'classical' cadherins, which mediate adhesion via *trans* homodimerization between their membrane-distal EC1 domains that extend from apposed cells, and assemble intercellular adherens junctions through *cis* clustering. To form mature *trans* adhesive dimers, cadherin domains from apposed cells dimerize in a 'strand-swapped' conformation. This occurs in a two-step binding process involving a fast-binding intermediate called the 'X-dimer'. *Trans* dimers are less flexible than cadherin monomers, a factor that drives junction assembly following cell–cell contact by reducing the entropic cost associated with the formation of lateral *cis* oligomers. Cadherins outside the classical subfamily appear to have evolved distinct adhesive mechanisms that are only now beginning to be understood.

The classical cadherin family

Cadherins constitute a large superfamily of cell surface receptors, many of which function in calcium-dependent cell–cell recognition and adhesion. Cadherins are found in a wide array of species ranging from unicellular animals with multicellular life stages [1,2] to mammals, in which they are involved in morphogenetic processes such as embryonic cell layer separation, synapse formation and specificity in the central nervous system [3,4], mechanotransduction [5,6], cell signaling [7,8], and physical homeostasis of mature tissues [9,10]. Consistent with these roles, decreased cadherin expression, which may allow cells to escape normal viability requirements for cellular cohesion [11–13], is a common feature of metastasis.

Members of the cadherin superfamily are defined by a common structural component, the EC domain – an approximately 110 residue β-fold domain – and cadherins can be classified into multiple subfamilies based on the number and arrangement of EC domains (Box 1, Figure I). By far the best understood of these subfamilies are the vertebrate classical cadherins, comprising six 'type I' and 13 'type II' cadherins in typical vertebrate genomes, which share a conserved cytoplasmic domain and an ectodomain containing five tandem EC domains (Figure 1). Linkers between successive EC domains are each stabilized by the binding of three Ca^{2+} ions resulting in a characteristic curvature of the ectodomain (Figure 1). The role of Ca^{2+} binding in classical cadherin mediated adhesion is summarized in Box 2.

Classical cadherins provide the prototypical example of calcium-dependent homophilic cell–cell adhesion. They are often concentrated at adherens junctions (reviewed in [14]), specialized cell–cell adhesion structures characterized by parallel apposed plasma membranes with an intermembrane space of approximately 15–30 nm. In these junctions, cadherins form *trans* bonds bridging the intermembrane space via their ectodomains, while their cytoplasmic domains bind to the adaptor proteins β-catenin, which links cadherins indirectly to the cytoskeleton (reviewed in [8]), and p120 catenin which regulates cadherin turnover and modulates actin assembly (reviewed [15,16]).

Recent studies suggest that the ectodomains of classical cadherins, in the absence of cytoplasmic regions, are sufficient to drive the initial assembly of adherens junctions [17–19]. This process is mediated by cooperative formation of distinct cadherin–cadherin interfaces in *cis* (on the same cell) and in *trans* (on different cells). These prototypical interfaces of classical cadherins, and their roles in adhesion, are described in detail below. The molecular mechanisms of non-classical cadherins are less clear; recent structural and functional insights into this diverse group of proteins suggest various ectodomain interactions beyond our current knowledge.

Extracellular cadherin domains drive adhesion from outside the cell

The relative contributions to adhesion of the extracellular and intracellular regions of classical cadherins are only now becoming clear. Early cell adhesion studies using cadherins engineered to lack p120 and β-catenin binding sites in the cytoplasmic domain demonstrated loss of adhesion, initially leading to the conclusion that the cytoplasmic machinery is essential for cadherin clustering and junction formation [20]. However, a recent study using E-cadherin similarly lacking the β-catenin and p120 binding sites but, crucially, with an endocytic clathrin adapter binding motif also deleted, showed effective junction formation [17,18]. In A431

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cells, which have background expression of wild-type E-cadherin, these 'tail-less' cadherins are effectively recruited into wild-type adherens junctions [18]. In addition, in cadherin-deficient A431-D cells, the tail-less cadherins form clusters at cell contact sites that closely resemble wild-type adherens junctions observed in the same study [18]. Similarly, in MDCK II cells and other epithelial cell lines, transfected catenin-uncoupled E-, N- and VE-cadherins [19] were also found to form adherens junction-like clusters in the lateral membranes. These results suggest that the cytoplasmic region is dispensable for the initial assembly of adherens junctions, yet to visualize these junctions in cells requires the uncoupling of endocytosis so that cadherin cell surface lifetimes are increased [17,18].

To test the hypothesis that classical cadherin extracellular domains can self-assemble to form junction-like structures, two groups separately developed cell-free liposome systems in which cadherin ectodomains bound to the liposome surface were assessed for adhesion and junction formation [17,21]. Cryo-electron microscopy (cryo-EM) revealed that both liposome-attached E-cadherin [17] and VE-cadherin [21] extracellular domains clustered at sites of adhesive contact between liposomes and formed 'artificial adherens junctions' characterized by dense cadherin clustering and flattening of the apposed membranes [17]. Taken together, these cellular and biophysical studies demonstrate that vertebrate classical cadherin extracellular domains are sufficient to form initial cell–cell contacts and assemble adherens junction-like structures even without contributions from the cytoplasmic machinery. Cytoplasmic interactions in adherens junction formation, such as stabilization of junctions by actin fiber recruitment (reviewed in [14,22]), are likely to function downstream of these initial extracellular events.

Mechanism of adhesive binding between single cadherin molecules from apposed cells

Classical cadherin ectodomains protrude from opposing cell surfaces and form *trans* adhesive homodimers through their membrane-distal EC1 domains, bridging the intermembrane space between neighboring cells (Figure 2a). The interface underlying this interaction has been characterized in detail from atomic resolution structures [17,23–27], revealing that all classical cadherins share a common binding mechanism in which the most N-terminal portion of the β-A strand, the A* strand, is swapped between EC1 domains of the adhesive partner protomers (Figure 2a). Key to this mechanism is the docking of conserved hydrophobic anchor residues located on the A* strand – tryptophan at position 2 (Trp2) for type I cadherins and Trp2 and Trp4 in type II cadherins – into a conserved hydrophobic pocket in the body of the partnering EC1 domain. The physiological relevance of this 'strand-swapped' adhesive interface has been confirmed in numerous mutation, electron microscopy, structural and cell studies [8,14,28–30].

The exchange of β-strands observed in classical cadherins is an example of the '3D domain swapping' protein interaction mechanism [31] in which the swapping domain (the A*-strand) can dock into its own pocket to form a 'closed' monomer (Figure 2b, left panel) or can dock into the pocket of the partner EC1 domain to form a swapped dimer (Figure 2b, right panel). A necessary step in the transition of the closed monomer to the swapped dimer is an open monomer state in which the swapping domain, the A* strand, is undocked, allowing dimer formation between two open monomers. Notably, the swapping domain is located in a closely similar residue environment in the 'closed' monomer and swapped dimer states. Therefore, the closed monomer form can be thought of as a competitive inhibitor for the swapped dimer. This competition is

Box 1. Meeting the family

Cadherins are membrane associated glycoproteins, many of which function in calcium-dependent cell adhesion or recognition processes. Each EC domain comprises a seven stranded β-barrel [23–26,30,51,65,70,71,75,80] with the N and C termini located on opposite sides allowing consecutive domains to be arranged in tandem. Most EC domains contain conserved Ca^{2+}-binding sites that coordinate three Ca^{2+} ions in the linker regions between consecutive domains [24], rigidifying the ectodomain structure [81] and protecting it from proteolysis [82] (Box 2). Less frequently, and mostly in very long cadherins, canonical EC domains can lack Ca^{2+}-binding residues resulting in Ca^{2+}-free linker regions, suggesting flexibility that could result in more globular overall structures [2,75]. The number of EC domains, overall domain organization and other sequence characteristics vary widely between different cadherins, dividing the superfamily into several subfamilies (Figure I) [83,84]. Vertebrate classical type I and type II cadherins are single-pass transmembrane proteins with ectodomains comprising five EC repeats (after removal of the N-terminal prodomain), and a short, highly conserved cytoplasmic domain with binding motifs for the armadillo proteins p120 and β-catenin (reviewed in [8]). Desmosomal cadherins, expressed in all vertebrate animals, have a domain organization similar to that of classical cadherins (reviewed in [60]). However, they are attached via distinct cytoplasmic proteins to intermediate filaments forming specialized cell–cell junctions, referred to as desmosomes, in tissues exposed to high mechanical stress. The largest cadherin subfamily is the protocadherins, divided into the gene-clustered α-, β- and γ- and non-clustered protocadherins (reviewed in [85,86], respectively). They are single-pass transmembrane proteins with six or seven EC domains and distinct cytoplasmic domains, and are expressed primarily in the nervous system of mammals. Clustered protocadherins are thought to play an important role in neural patterning [66,85]. Other subfamilies are more divergent; for example, Flamingo/CELSR cadherins, which mediate planar cell polarity in vertebrates and invertebrates, have nine EC repeats, EGF, laminin-G like and hormone receptor-like domains and, uniquely in the cadherin family, a seven-pass transmembrane structure [87]. Some atypical cadherins, such as FAT and Dachsous, are involved in adhesion-mediated signaling and planar cell polarity [88]. These cadherins have many EC domains, but have a close phylogenetic sequence resemblance to protocadherins in their N-terminal region [2]. Invertebrate 'classical' cadherins, typified by *Drosophila* N- and E-cadherin, are found in adherens junction-like structures but deviate greatly in their domain organization. The heterophilic adhesive pair cadherin-23 and protocadherin-15 appear to form a long braided structure, the 'tip-link', which is involved in auditory mechanotransduction [6,89]. Each vertebrate genome contains a solitary truncated (T-) cadherin [90], which regulates neurite outgrowth and has a similar overall domain organization to the ectodomain of classical cadherins, but the transmembrane and cytoplasmic domain are replaced by a GPI anchor. T-cadherin binds adhesively through the 'X-dimer' interface [51], which functions as a binding intermediate in vertebrate classical cadherins [48].

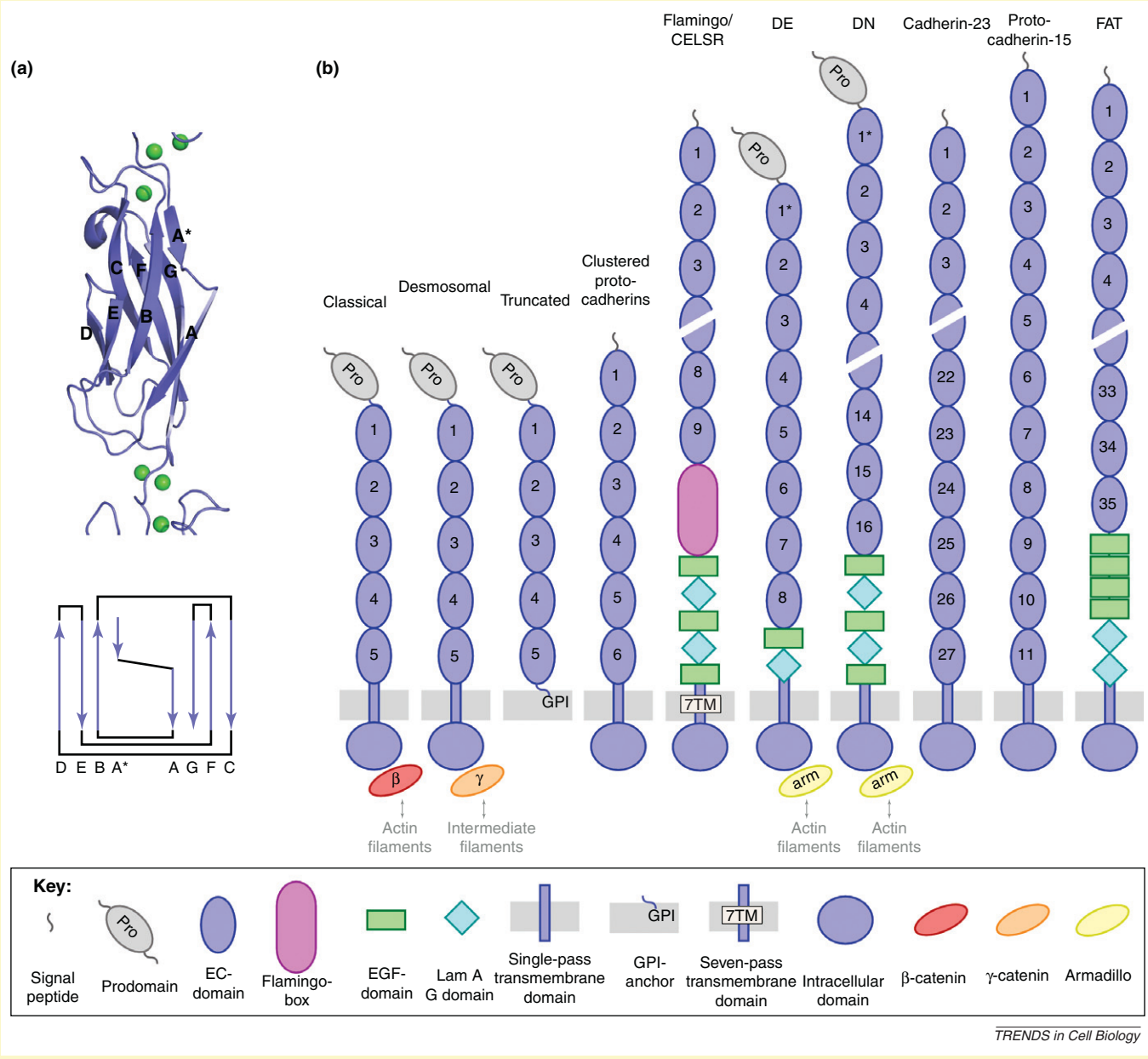

Figure I. Schematic representation of members of the cadherin family, which share a common structural motif: the EC domain. (**a**) Typical folding of an EC domain shown in ribbon representation (top panel from pdb-ID: 1L3W). Seven antiparallel β-strands (A–G) assemble two β-sheets as shown in the topology diagram (lower panel). Note that the A strand is split into two halves, the A* and A strands. These are connected by a loop, referred to as the 'hinge'. Three Ca^{2+} ions (green spheres) are coordinated between consecutive EC domains. (**b**) Schematic representation of overall domain organization of selected cadherin family members. All cadherins have two or more EC domains in their extracellular regions (blue ovals, numbered from membrane distal to membrane proximal domain), which can also contain non-EC domains such as EGF-repeats (green rectangles), laminin A G domains (cyan diamonds) and flamingo boxes (pink oval). Some cadherins have, in addition to the signal peptide, a prodomain (grey ovals) that is removed by a furin protease on the cell surface. Hashed domains indicate four or more EC domains omitted for clarity. *The first EC domain of *Drosophila* E- (DE) and N- (DN) cadherin is predicted from sequence analysis [75].

responsible for the weak binding affinities of classical cadherins [32], and requires that structural differences exist that stabilize the dimer and/or destabilize the monomer to drive dimerization.

A comparison of cadherin domains that engage in strand swapping (the EC1 domains of classical cadherins) with non-swapping cadherin domains (EC2–5) identified numerous factors that favor the formation of strand-swapped dimers [33]. Swapping cadherin domains were found to have a shortened β-A strand, in addition to the conserved tryptophan at position 2, which is replaced by a phenylalanine in other EC domains. A glutamic acid residue (Glu11) at the base of the A strand coordinates Ca^{2+} in all classical cadherins, and anchoring of the A strand at both ends – at the base by Ca^{2+} binding to Glu11 and at the N terminus by Trp2 docking – induces strain in the shortened A strand. This in turn destabilizes the closed monomer and thus favors swapped dimer formation, in which this strain is released [34–36]. A naturally monomeric non-swapping EC2 domain was successfully 'converted' into a strand swap-binding EC1-like domain by introducing point mutations, thereby validating this mechanism [34].

Interestingly, although strain in the 'closed' monomer favors swapped dimer formation, selective pressure also

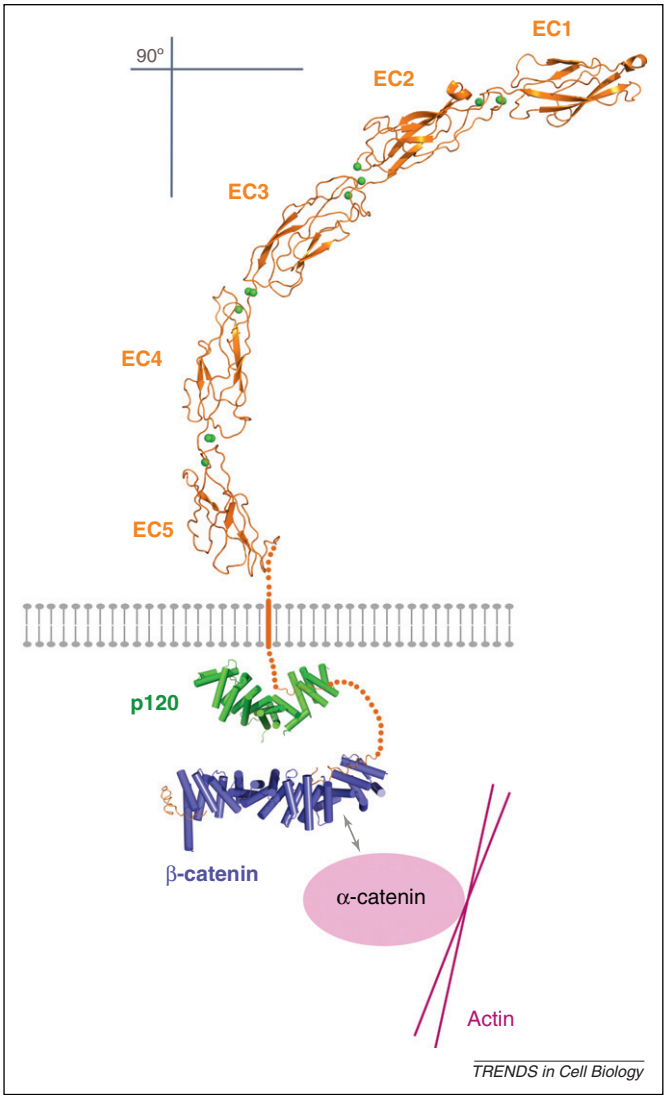

Figure 1. Overall architecture of classical cadherins. The extracellular domain of C-cadherin (pdb-ID: 1L3W) is depicted as a ribbon diagram (orange). Ca^{2+} ions (green spheres) are coordinated between consecutive domains, stabilizing an overall curved shape of the ectodomain, with an angle of close to 90° between domains EC1 and EC5. The structure of the stalk region, the transmembrane domain and parts of the intracellular domain are unknown and are shown as dotted lines. The cytoplasmic domain of cadherins binds to intracellular binding partners p120 (green barrels representing α-helices; pdb-ID: 3L6X) in the juxta-membrane region and β-catenin (blue barrels representing α-helices; pdb-ID: 1I7X) in the C-terminal region. β-catenin interacts with α-catenin, which in turn binds to actin filaments linking cadherins to the cytoskeleton. The depicted orientation, position and size of the intracellular binding partners relative to each other and to C-cadherin are schematic; the overall structural arrangement of the cytoplasmic side of adherens junctions is unknown.

appears to have kept adhesive binding weak. Type I classical cadherin EC1 sequences include a conserved Pro5-Pro6 motif that prevents continuous β-sheet hydrogen bonding between cadherin EC1 domains of adhesive dimers. When the diproline motif is mutated to alanine in E- and N-cadherins, dimer affinity is enhanced [34] and, as opposed to their wild-type counterparts [37] (see below), the mutant N- and E-cadherin dimerization affinities become indistinguishable. Crystal structures of these mutants reveal continuous β-strand hydrogen bonds between the A strands of partner EC1 domains, explaining the loss of binding specificity [34]. The diproline motif thus appears to be a required structural element underlying the differential binding affinities of N- and E-cadherin.

All vertebrate classical cadherins utilize a similar strand-swapping mechanism to form adhesive dimers; however, the interfaces found in the crystal structures of type I and type II cadherins are different (Figure 2c). The adhesive interface of type I cadherins is restricted to the pocket region near the apex of EC1 (Figure 2c, left panel) and the partner A* strand region, which includes the anchoring tryptophan residue Trp2. By contrast, in type II cadherins, two tryptophan residues, Trp2 and Trp4, are swapped. Moreover, the dimer interface in type II family members extends along the entire face of the EC1 domain involving conserved hydrophobic residues at position 8, 10 and 13 (Figure 2c, middle panel) [26]. Interestingly, VE-cadherin, a divergent classical cadherin and the crucial adhesion protein of the vascular endothelium [38], blurs the definition between type I and type II cadherin interfaces. In common with type II cadherins, VE-cadherin docks Trp2 and Trp4 into the hydrophobic pocket of its partner, but lacks the hydrophobic interactions along the rest of the EC1 domain (Figure 2c, right panel) and thus has an overall dimer arrangement more similar to that of type I cadherins [27].

Classical cadherin homophilic binding specificity at the cellular level is governed by EC1, as shown in domain shuffling experiments [26,39–42], suggesting that differences in the strand-swapping interface modulate specificity.

Box 2. Calcium dependence of cadherin adhesion

Cadherins are named for the dependence of their adhesive function on the presence of extracellular calcium. Before their structures were known, it was speculated that Ca^{2+} ions might bridge the adhesive interface. However, the role of calcium in cadherin function is far more complex. Calcium binds to cadherins at stereotyped binding sites situated between successive EC domains. Each of these sites binds three Ca^{2+} ions in a highly cooperative manner such that each five-domain classical cadherin coordinates twelve Ca^{2+} ions in total [17,24,80]. The binding affinities of the Ca^{2+} sites vary, but all bind with a dissociation constant (K_D) lower than the Ca^{2+} concentration characteristic of the extracellular milieu, approximately 1 mM [91,92]. Thus, it is expected that cadherin ectodomains will be fully Ca^{2+}-occupied under physiological conditions.

Three roles are now understood for Ca^{2+} binding in classical cadherins. The first is rigidifying the ectodomain so that it adopts a characteristic crescent shape [81], although this structure retains considerable conformational flexibility [55,70]. The crescent shape is critical to adhesive binding because the axes of the membrane-distal and membrane-proximal EC domains must be approximately 90° apart to satisfy the geometrical requirements of *trans* binding [17,24]. Notably, chelation of Ca^{2+} leads to the loss of *trans* binding and its concomitant replacement by binding to other cadherins on the same cell through the adhesive interface [93]. Thus, Ca^{2+}-mediated rigidification is critical to adhesive *trans* binding.

A second role for Ca^{2+} ions is in defining the structure of the X-dimer interface surfaces. The X-dimer binding intermediate of classical cadherins is centered around the EC1–EC2 Ca^{2+} binding region, which is unstructured in the absence of Ca^{2+} [48,51,80,94,95]. Thus, in the absence of Ca^{2+}, the mature adhesive strand-swap interface is likely to be kinetically unfavorable due to the slow exchange inherent in domain swap binding.

The third role for Ca^{2+} involves direct energetic effects on strand swapping. NMR experiments [46] and molecular simulations [96] reveal that Ca^{2+} ligation favors the opening of the A strand. The underlying molecular mechanism has recently been described [34] and is discussed in the text.

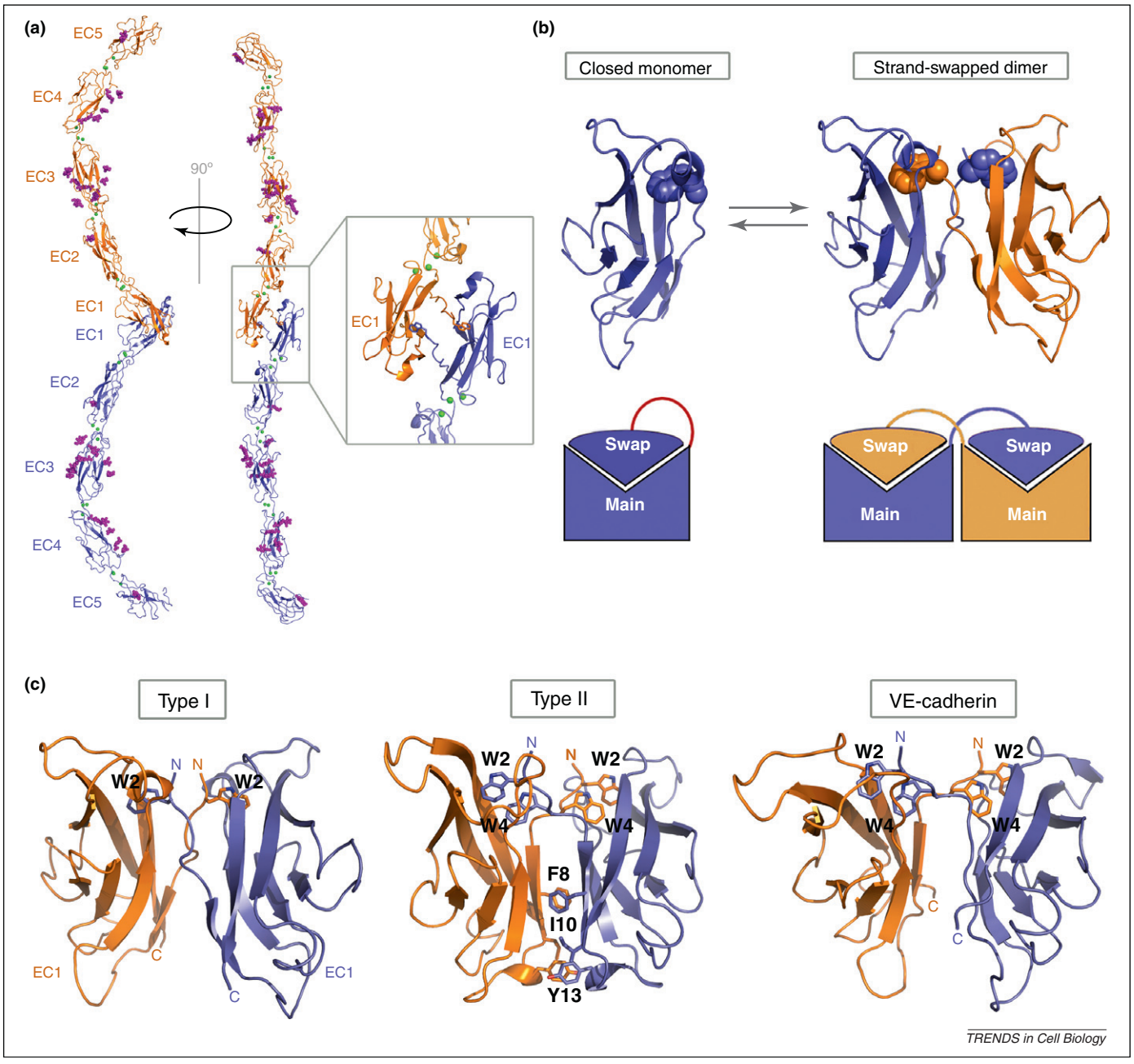

Figure 2. Classical cadherins from adhesive dimers by exchange of the N-terminal β-strand. (**a**) A classical cadherin *trans* dimer is shown as a ribbon diagram in two orthogonal orientations; one protomer is shown in blue, one in orange (from pdb-ID: 3Q2W). Membrane distal EC1 domains overlap and exchange N-terminal β-strands (expanded view). Note that substantial O- and N-linked glycosylation (magenta and green spheres, respectively) is found on extracellular domains on EC2–4, but not on adhesive EC1 domains. Ca^{2+} ions are shown as green spheres. (**b**) The adhesive mechanism of classical cadherins is an example of 3D domain swapping. EC1 domains are shown for the monomer and the dimer (ribbon representation). The swapping element, residue Trp2 (side chain depicted as spheres), has an identical residue environment in the monomer (left panel) and the 'strand-swapped' dimer (right panel). Adapted with permission from [8]. (**c**) Ribbon presentations of strand-swapped EC1 domains of type I E-cadherin (pdb-ID: 2QVF), type II cadherin-11 (pdb-ID: 2A4E) and VE-cadherin (pdb-ID: 3PPE). Residues characteristic of the adhesive interfaces of each subfamily are depicted as sticks. In type I cadherins, residue Trp2 in domain EC1 is swapped between binding partners. In type II cadherins, two Trp residues, Trp2 and Trp4, are exchanged, and, in addition, hydrophobic interactions occur between conserved residues Phe8, Ile10 and Tyr13 giving rise to an extended interface. VE-cadherin exchanges Trp2 and Trp4 like type II cadherins, but the interface is limited to the apex of the domain, as in type I cadherins.

Type I cadherins in general do not bind to type II cadherins [8,26,37,43] consistent with the substantial differences in the canonical adhesive interface structures of each subfamily. Interestingly, classical cadherins interact promiscuously within subfamilies, consistent with the close similarity in the interface region between individual members [26,37,43,44]. Thus, within subfamilies, cadherins exhibit both homophilic and heterophilic binding properties, which combine to yield the homophilic aggregation behavior of cadherin-expressing cells [37].

Speed dating: the X-dimer intermediate

Formation of strand-swapped dimers requires refolding of each partner protomer to transition from the 'closed' monomer form (Figure 2b, left panel) to the 'open' dimer form (Figure 2b, right panel). This conformational change could render dimerization kinetically unfavorable because, this interconversion can occur over long periods of time in other proteins that engage in 3D domain swapping [31], yet binding is fast for cadherins [35,37,45]. Two alternative mechanisms have been proposed to explain the kinetics of

cadherin interaction: 'selected fit', in which cadherin monomers exist in equilibrium between open and closed forms and dimerization results from collision of two open monomers; and 'induced fit', in which cadherin monomers first form a non-swapped intermediate – an 'encounter complex' – that lowers the activation energy required for strand swapping to occur [46]. Recently, single-molecule fluorescence resonance energy transfer (FRET) experiments provided evidence for an encounter complex in E-cadherin, strongly favoring the induced fit pathway for classical cadherin mediated interaction [47]. When strand swapping was ablated by a Trp2 to Ala mutation, dimers still formed between EC1 domains, with slightly altered FRET distances compared with swapped dimers, suggesting the existence of a non-swapped dimer form. Additionally, atomic force microscope (AFM) experiments showed the non-swapped mutant dimers to be weaker than strand-swapped wild-type dimers, energetically consistent with a role as a binding intermediate [47].

Crystallographic studies of strand swap-impaired cadherin mutants revealed the molecular details of this encounter complex [48]. For numerous swapping-incompetent mutants, a dimer with its interface centered around the EC1–EC2 interdomain linker region and the apex of EC2 (Figure 3, middle panel) is observed. This structure is now referred to as the 'X-dimer' because the relative orientation of the interacting protomers is reminiscent of an 'X' shape (Figure 3). The X-dimer requires no refolding for its interaction, enabling fast binding kinetics. Importantly, the X-dimer positions the A strands of each protomer parallel to each other in close proximity as if poised to swap [48]. A similar structure was observed in a strand swap-deficient mutant of type II cadherin-6 [48].

The role of the X-dimer as an encounter complex is confirmed by the observation that mutations designed to prevent X-dimer formation, while leaving strand swapping intact, significantly slowed the binding rate of E-cadherin and cadherin-6. Specifically, no dimerization is observed in short term SPR assays, but there is no loss of affinity in long term analytical ultracentrifugation experiments [48]. Furthermore, unlike wild-type proteins, X-dimer mutant monomers and dimers could be resolved as stable monomer and dimer species in size exclusion chromatography and velocity ultracentrifugation experiments, indicating slow exchange rates between these two forms [48]. In unpublished work, we find a similar structure and binding behavior for the X-dimer of N-cadherin and, in addition, mutation of the predicted X-dimer interface in N-cadherin has been shown to abolish cell–cell aggregation activity [49]. Similar to the encounter complex observed via FRET experiments, X-dimers were found to have weaker binding affinity than wild-type swapped dimers [48]. In transfected epithelial cells, cadherin X-dimer mutants formed extraordinarily stable cell–cell junctions [50], consistent with slowed monomer–dimer exchange rates observed in cell free experiments [48], although effects on dimer dissociation were emphasized by the authors. Taken together, current data favor a mechanism in which the X-dimer functions as an intermediate in the formation and disassembly of the 'mature' adhesive dimer. The structural and functional observation of X-dimers in type I E-cadherin and the relatively distant type II cadherin-6 (34% identity over EC1–EC2), together with sequence conservation patterns of interfacial residues [17], suggests that the X-dimer mechanism may be general among members of the two subfamilies of vertebrate classical cadherins.

Interestingly, T-cadherin, a divergent classical cadherin anchored to the plasma membrane via a glycosylphosphatidyl inositol (GPI) anchor (Box 1), does not strand swap and adopts an X-dimer conformation for its mature adhesive binding interface [51]. Mutations targeting the X-dimer interface in T-cadherin were found to abolish its function in modulation of neurite outgrowth, whereas targeted strand dimer mutations, analogous to those that abolish strand-swap binding in classical cadherins, had no effect on T-cadherin function or homodimerization [51]. The close phylogenetic relation to type I classical cadherins suggests that T-cadherin represents a classical cadherin that has lost its swapping ability. Other roles for X-dimers outside the classical cadherin subfamily remain unknown.

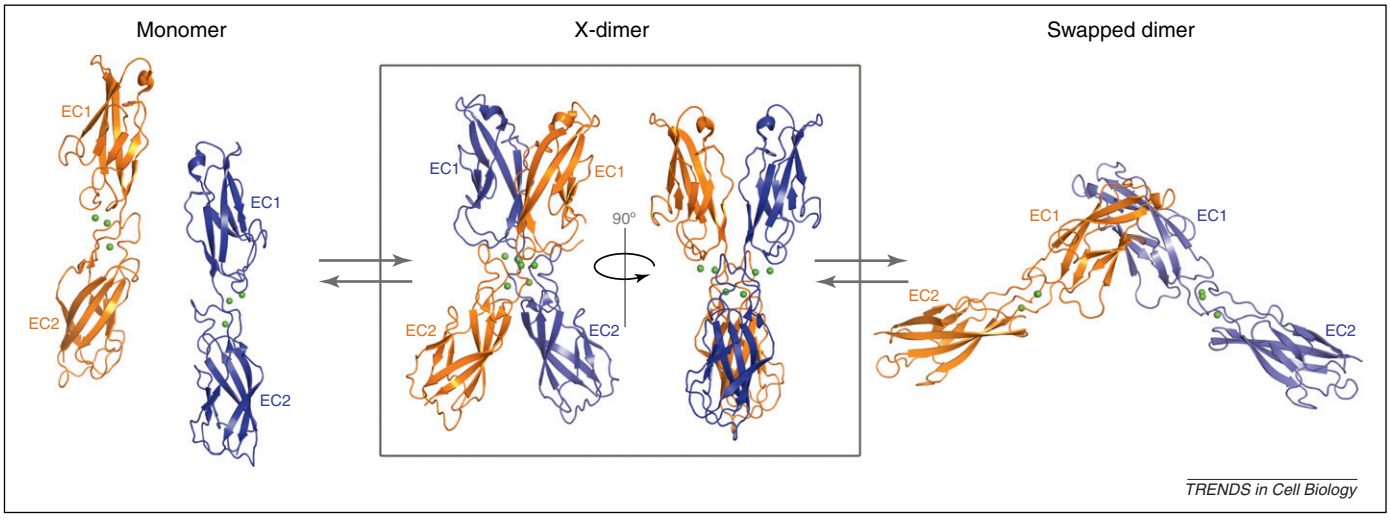

Figure 3. Strand-swapped adhesive dimers of classical cadherins form through a non-swapped intermediate. E-cadherin monomers (orange and blue ribbon diagrams (left panel); only EC1–2 shown for clarity) associate via an 'X-dimer' interface in which N-terminal strands are not swapped but are closely apposed (middle panel). Swapping of strands leads to formation of mature strand-swapped dimers (right panel). Assembly and disassembly of swapped dimers is likely to proceed via the same pathway. Protomers shown as orange and blue ribbon diagrams with only EC1–2 domains shown for clarity.

From bonds to junctions

Cell–cell adhesion in mature tissues is mediated in part by adherens junctions where numerous cadherin *trans* dimers assemble. In principle, a passive diffusion trap mechanism, whereby cadherins would become concentrated at cell–cell contact sites through their adhesive interactions, could explain the accumulation of cadherins at sites of intercellular contact [52]. However, mutations in a crucial *cis* interface (described below; Figure 4a) which leave adhesive binding intact show that the diffusion trap mechanism is insufficient to achieve the level of concentration at cell–cell contacts observed for wild-type cadherins [17]. It therefore appears likely that lateral or *cis* interactions could account for the enhanced localization of classical cadherins at cell contact sites.

A potential lateral interaction site, apparently conserved among type I cadherins, has been observed in crystal structures of full-length ectodomains of C- [24], N- and E-cadherins [17]. Despite forming crystals that are unrelated to one another, in addition to the adhesive strand-swap interface, all three structures reveal a lateral interface formed between the base of the EC1 domain of one protomer and a region near the apex of EC2 of a parallel partner (Figure 4a). The combination of *cis* and *trans* interactions engaged by each cadherin molecule (Figure 4b) creates a molecular layer within each crystal that is likely to correspond to the extracellular structure of the adherens junction [17,24]. The region of EC1 involved in this *cis* interface is opposite to the strand-swapping site, so that *cis* and *trans* interactions can form simultaneously resulting in a continuous two-dimensional lattice with dimensions near to that expected for adherens junctions (Figure 4c).

When cadherin ectodomains are bound to the surface of liposomes, in the absence of other proteins, cryo-EM analysis reveals ordered junction-like structures that resemble the molecular layer observed in C- [24], E- and N-cadherin crystals [17]. This system, as well as cell-based experiments, was used to test the idea that the *cis* interface underlies the lateral assembly of cadherins in adherens junctions. Mutations that targeted the *cis* interface of E-cadherin (without interfering with *trans* strand-swapped dimerization) still allowed a reduced level of adhesion between liposomes; however, the ordered structure of the reconstituted junctions was lost [17]. Consistently, incorporation of these mutants into endogenous wild-type cellular junctions caused these junctions to become unstable and transient [17]. In cells lacking endogenous cadherin, *cis* mutant protein localized to sites of cell contact but failed to cluster into junction-like structures [17]. Taken together, these data suggest that the *cis* interface identified in structural studies functions to laterally assemble cadherin *trans* dimers into adherens junctions. *cis* oligomerization of cadherins at adherens junctions might account for previous observations of multiple adhesive states between cadherin monolayers in molecular force experiments [45,53,54] that were initially interpreted as multiple *trans* dimer states, but could be explained by combinations of *cis* and *trans* interactions.

Interestingly, the *cis* interaction is too weak to be detected in solution binding experiments (which are limited to a detection level of approximately 1 mM) [17], yet as discussed above it appears to play a crucial biological role. This is not surprising because the strength of interaction between proteins in solution can differ significantly from that of the same interaction in the context of restricted motion when membrane bound [55]. Indeed, *in silico* simulations suggest that when *trans* ectodomain dimers form, flexibility is dramatically reduced because the two interacting protomers are now attached to each other via the adhesive interface, and in addition are tethered to each of the apposed cell membranes [55]. Thus, when *trans* dimers are formed, conformational flexibility is decreased, which lowers the entropic penalty associated with *cis* dimer formation [55,56]. This model, in which *cis* assembly requires *trans* dimerization, would account for observations that cadherins do not cluster in the absence of cognate adhesion to an apposed cadherin-expressing cell [12,13].

Large cellular adherens junctions such as the zonula adherens that circumscribe epithelial cells appear less dense than desmosomes (see below) and it is possible that they are assembled from numerous subdomains, each with the defined layer structure described above. The cadherin extracellular lattice structure is directional such that two such subdomains would have to meet with an appropriate

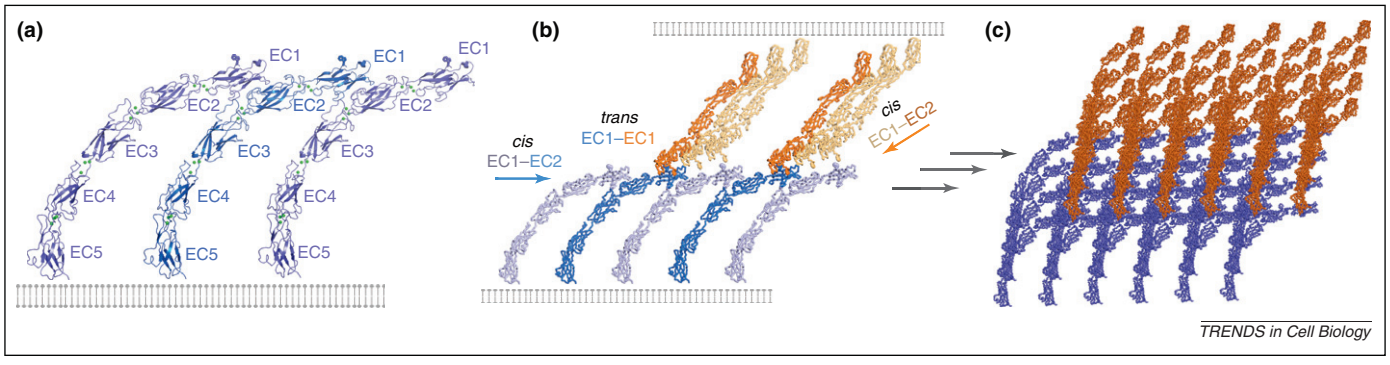

Figure 4. Extracellular structure of adherens junctions formed through *cis* and *trans* ectodomain interactions. (**a**) Selected region of the N-cadherin EC1–5 crystal lattice (blue ribbon presentation; pdb-ID: 3Q2W) showing an array of N-cadherin molecules oriented as if emanating from the same cell membrane and connected by a *cis* interface formed between the EC1 and EC2 domains of neighboring molecules. (**b**) Strand-swapped *trans* dimers form together with *cis* interactions in the same crystal lattice. *trans* interactions orient opposing *cis* arrays approximately perpendicularly such that each *cis* array (blue) forms *trans* interactions with multiple opposing *cis* arrays (orange). (**c**) The combination of *cis* and *trans* interactions enables cadherin ectodomains to form an ordered network that is thought to be the basis for the extracellular architecture of adherens junction. Adapted from [17].

orientation to achieve a continuous merger. Whether zonula adherens are formed from continuous structures or collections of defined puncta is not clear from current data. Maturation of these structures requires cytoskeletal activity, which could play a role in driving their assembly from smaller puncta [57]. Currently, we favor the view that small punctate clusters are likely to auto-assemble via their ectodomains when two cognate cadherin-expressing cells come into contact, and these may later be incorporated into mature large adherens junctions by cytoplasmic processes. Further investigation will be needed to elucidate the interplay between extracellular and cytoplasmic mechanisms in cadherin assembly.

Although type II classical cadherins have the same adhesive mechanism as type I cadherins, the *cis* interface described above has not been found in any of the multidomain crystal structures of type II cadherins [26,27,48]. Nonetheless, there is evidence that at least some members of the type II cadherin family may form adherens junction-like structures. In particular, junctions mediated by the divergent VE-cadherin appear similar to those of type I cadherins seen by EM [58]. Cadherin-11, a type II cadherin, has also been observed to colocalize with p120-catenin, α-catenin and actin filaments at cell–cell contacts [59], but it remains to be determined whether cadherin-11 and other type II cadherins form junctions with ultrastructures similar to those observed for type I cadherins. Thus, VE-cadherin and possibly other type II cadherins may form cell–cell junctions via a different lateral interface yet to be determined.

Non-classical cadherin subfamilies suggest diversity of adhesive mechanism

Desmosomal cadherins

Sequence conservation analyses suggest that desmosomal cadherins (Box 1, reviewed in [60]), the major component of desmosome junctions, also adhere through a strand-swap binding mechanism. These cadherins have the classical Trp residue conserved at position 2, and hydrophobic residues corresponding to the Trp binding pocket in classical cadherins [33,60]. Moreover, mutation of Trp2 or the hydrophobic pocket abolishes *trans* binding of desmocollin 2 in cross-linking experiments [61]. A nuclear magnetic resonance (NMR) structure of an EC1 fragment of human desmoglein-2 (pdb-ID: 2YQG) shows a domain fold remarkably similar to type I classical cadherins. This structure is monomeric and Trp2 is self-docked, probably owing to the inclusion of ten residues preceding the native N terminus due to cloning artifacts. Similar extensions are known to inhibit strand-swap dimerization in classical cadherins [25,48].

Cryo-electron tomography of vitreous sections of desmosomes from human skin [62] and electron tomography of mouse skin sections embedded in plastic [63] show an extracellular arrangement compatible with *trans* dimerization via EC1 domains. Desmosomes in human skin showed a highly ordered arrangement in the extracellular region, whereas those of mouse skin were relatively disordered, probably due to differences in sample preparation. Fitting of structures of C-cadherin ectodomains [24] into a 34 Å resolution cryo-EM map of desmosomes in human skin suggested a molecular array comprising a linear 'zipper' formed from alternating EC1-mediated *cis*- and *trans*-interactions [62] distinct from the two-dimensional array observed for type I classical cadherins [17,24]. Alternatively, the possibility that desmosomal cadherins form similar assemblies to classical cadherins has also been suggested, based on EM of lanthanide infiltrated desmosomes from guinea pig heart [64]. Because an atomic resolution structure of *cis* and *trans* dimers of desmosomal cadherins is not yet available, further mutational and structural studies are needed to reveal their detailed binding mechanism. Interestingly, binding interactions between desmogleins and desmocollins have also been shown to display a high degree of isoform specificity [61].

Clustered protocadherins

Clustered protocadherins, named because they are encoded in three novel gene clusters (α, β and γ) are predominantly expressed in vertebrate brain (Box 1) and constitute the largest cadherin subfamily. However, their adhesive properties are poorly understood. Numerous single domain structures have been determined for protocadherins (pdb ID: 2EE0, 2YST, 1WYJ, 1WUZ; [65]), but none appear to include a functional adhesive binding site, which remains elusive. Transfected cell aggregation studies showed strict homophilic binding specificity for seven members of the protocadherin γ-cluster [66]. In the same system, domain shuffling experiments showed that consecutive domains EC1 through EC3 are crucial for *trans* adhesion and, interestingly, domains EC2 and EC3 were found to govern protocadherin specificity in cell aggregation assays [66]. Notably, these domains show the highest sequence diversity among individual protocadherin isoforms [66]. Since individual neurons express multiple protocadherin isoforms [67,68], homophilic specificity of this type could give rise to an enormous range of potential cellular affinities. It has further been suggested that multiple isoforms can associate as *cis* tetramers on the same cell surface to mediate combinatorial specificity [66], although this model remains untested.

Large cadherins with many EC domains

Numerous members of the cadherin superfamily – in both vertebrates and invertebrates – are large proteins containing many EC domains (Box 1). Although relatively little is known about their structure/function relations, early studies suggest that some of these proteins adopt extended conformations, whereas others may form structures more like folded globular 'superdomains'. Two atypical members of the superfamily that appear to adopt extended structures, cadherin-23 (27 EC domains) and protocadherin-15 (11 ECs) (Box 1 and Figure I) [69], link stereocilia of hair cells by formation of an extracellular structure, known as the tip-link, assembled by *trans* heterophilic interaction between *cis* homodimers [6]. Recently, the atomic resolution structure of an N-terminal EC1–2 domain fragment of cadherin-23 was determined [70,71] revealing a domain architecture closely similar to that of other known cadherins (Figure 5a), as well as features unique to cadherin-23 including a 3_{10} helix in the A strand, an α-helix between β strands C and D of EC1 and, notably, an additional novel

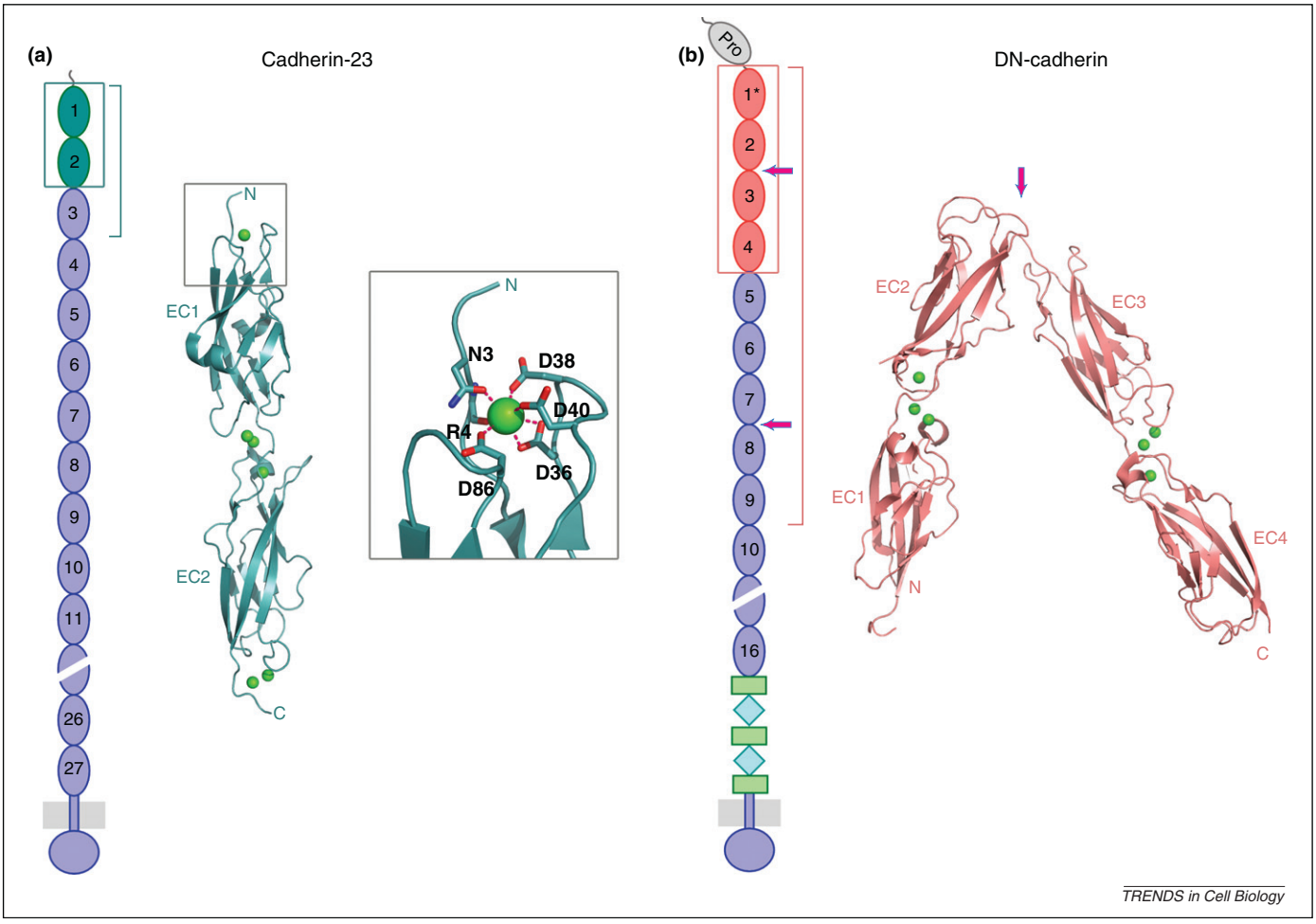

Figure 5. Crystal structures of cadherin-23 and *Drosophila* N-cadherin reveal unique features of atypical cadherins. (**a**) Structures of mouse cadherin-23 EC1–2, which are involved in adhesive binding to protocadherin-15 (binding domain indicated by brackets in schematic) reveal successive EC domains (ribbon diagram, pdb-ID: 3MVS, 2WHV) with three Ca^{2+} ions (green spheres) coordinated in the linker region. Uniquely, a Ca^{2+} binding site was identified at the apex of EC1 (box), referred to as Ca^{2+} binding site 0. Structural determination of a complex of cadherin-23 with protocadherin-15 will help to identify the heterophilic binding interface. (**b**) Structures of DN-cadherin EC1–4, which is part of the adhesive interface for homodimerization (EC1–9, bracket in schematic), reveal four consecutive EC domains (ribbon diagram). Interestingly, Ca^{2+} coordination was found only between domains EC1–2 and EC3–4 and not between EC2–3 (pink arrow). This Ca^{2+}-free linker introduces a 'kink' in the otherwise linear structure. Sequence analysis suggests a second occurrence of a Ca^{2+}-free linker between EC7 and EC8 in the ectodomain of DN-cadherin; this may contribute to folding of the 16 EC domains into a compact form within the intermembrane space of *Drosophila* adherens junctions.

calcium binding site, referred to as site 0, at the apex of EC1 [70,71]. N-terminal fragments of cadherin-23 and protocadherin-15 comprising EC1–3 are sufficient for *trans* heterophilic binding, but not for *cis* homodimerization [6,70,71]. The structures of the heterocomplex of cadherin-23 and protocadherin-15 and of longer ectodomain fragments are needed to identify the *trans* and *cis* interfaces that form the tip-link.

DN-cadherin and DE-cadherin in *Drosophila melanogaster* are invertebrate classical cadherin orthologs, in that they mediate Ca^{2+}-dependent cell–cell adhesion, have conserved armadillo binding domains in their cytoplasmic region [72], and form adherens junctions with intermembrane distances of 20–30 nm, similar to those of mammals [14,73,74]. However, the extracellular domain organization is very different from that of vertebrate classical cadherins: there are 8 and 16 sequence-predicted EC domains arrayed in tandem for DE- and DN-cadherin [75], respectively, followed by epidermal growth factor (EGF)-like and laminin-G domains (Box 1, Figure I). Structures of the N-terminal portion of DN-cadherin were recently determined [75] and revealed that a Ca^{2+}-free linker region between domains EC2 and EC3 results in an acute interdomain angle in all three crystal forms that causes the otherwise linear structure of EC1–4 to 'jackknife' (Figure 5b). Bioinformatics analysis finds that other long cadherins such as FAT, FAT-like, Dachsous and CELSR/Flamingo also contain interdomain linker regions that lack some or all of the residues required for Ca^{2+} binding [75]. These findings suggest that long cadherin ectodomains might fold onto themselves, resulting in a more compact arrangement compatible with the relatively narrow intermembrane distance of adherens junctions. It remains to be determined whether these Ca^{2+}-free linker regions allow for flexibility or whether they introduce a fixed bend as observed in the three crystal structures so far determined. Deletion mutagenesis has mapped the minimal adhesive binding site of DN-cadherin to the EC1–9 domain region. The apparent requirement for nine EC domains is remarkably different from the vertebrate classical cadherins, for which all adhesive contacts are formed through EC1–EC1 interactions. The jackknifed bend between domains EC3 and EC4 – and another predicted between EC7 and EC8 (Figure 5b) – is reminiscent of Dscam immunoglobulin

superfamily adhesion proteins, which fold into a super-domain platform that positions multiple immunoglobulin domains for engagement in adhesive binding [76].

Concluding remarks

The structural basis of the adhesive function of vertebrate classical cadherins is becoming increasingly clear. Adhesive binding between cells uses a *trans* strand-swapping mechanism that is enabled by a fast-binding intermediate, the X-dimer. Vertebrate classical cadherins on isolated cells diffuse freely in the plasma membrane, but when they are bound by cognate cadherins from a contacting apposed cell, *trans* binding lowers the entropic penalty for the formation of *cis* interactions, initiating lateral oligomerization. These early processes depend only on the properties of cadherin ectodomains, yet subsequent events such as junction strengthening clearly involve interactions of the cadherin cytoplasmic region with regulatory and cytoplasmic elements.

The picture is far less clear for other cadherin subfamilies. Only vertebrate desmosomal cadherins – close relatives of the classical subfamilies – contain sequence elements indicative of strand-swap binding. Other members of the cadherin superfamily, including all invertebrate cadherins, seem likely to engage in adhesive binding by other means, and may adopt diverse binding mechanisms. It is remarkable how many different cadherin–cadherin interfaces have already been discovered, revealing a surprising complexity in the interactions of classical cadherins. However, longer cadherins appear to form interfaces through surfaces not yet defined. Moreover, some cadherins are known to bind to other proteins and, although for some of these the structural basis is known (for example E-cadherin binding to NKLRG1 [77] and to internalin [78]), for others, such as integrins [79], we have little structural insight into how such binding occurs. It is clear that, at this stage, our structural understanding is limited to only a small portion of the wider cadherin universe, which appears to exploit the remarkable versatility of the cadherin fold in forming diverse sets of protein–protein interfaces. Progress in our understanding of classical cadherins emphasizes the utility of combining insights from structural, cell biological, biophysical and computational studies. The new mechanistic insights that have been inferred may be applicable to many other classes of adhesion receptors.

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