CADHERINS IN EMBRYONIC AND NEURAL MORPHOGENESIS

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Cadherins not only maintain the structural integrity of cells and tissues but also control a wide array of cellular behaviours. They are instrumental for cell and tissue polarization, and they regulate cell movements such as cell sorting, cell migration and cell rearrangements. Cadherins may also contribute to neurite outgrowth and pathfinding, and to synaptic specificity and modulation in the central nervous system.

GROWTH CONE
Exploratory tip of an extending neuronal process such as an axon.

IMMUNOGLOBULIN-TYPE ADHESION MOLECULES
Family of adhesion molecules characterized by the presence of immunoglobulin-like domains, which are also found in antibody molecules.

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In 1963, two publications appeared that focused attention on the adhesive mechanisms that contribute to embryonic morphogenesis and govern structural differentiation of the nervous system. Malcolm Steinberg laid the groundwork for the 'differential adhesion hypothesis', suggesting that the segregation or 'sorting out' of different embryonic cell types into separate tissues involves qualitative or quantitative differences in cell adhesion[1]. Roger Sperry proposed the 'chemoaffinity hypothesis', which holds that specific synaptic contacts form according to differences in the adhesive properties of individual GROWTH CONES and synapses[2]. Both hypotheses were the result of decades of experimental work, but were formulated before any adhesion molecules had been identified. It is now apparent that a large number of adhesion molecules exist that can be grouped into several superfamilies. The cadherin and IMMUNOGLOBULIN-TYPE ADHESION MOLECULES are the main groups of cell–cell adhesion receptors, whereas the integrins are the predominant contributors to cell–substrate adhesion[3,4]. Adhesive mechanisms that contribute to embryonic or neural morphogenesis share many similarities, revealing that Steinberg's and Sperry's hypotheses are essentially similar proposals applied to different cell populations.

Morphogenesis involves two interrelated themes: structure and movement. For example, the different adhesive properties of two mixed cell populations induce cell movement, leading to the sorting out of the two groups of cells. After the sorting process is completed, adhesive differences maintain the segregation and relative position of the two cell groups, therefore preserving a specific tissue architecture[5–8]. Neuronal morphogenesis follows a similar pattern, in which the neuronal growth cone has to move through a complex environment using differential adhesive cues. On reaching the target, synaptic contacts are formed and maintained by specific adhesive interactions[9].

The first cadherins to draw scientists' attention were vertebrate classic cadherins, which were independently identified for their ability to mediate calcium-dependent adhesion among cultured cells and for their role in the epithelialization of the early mouse embryo[10]. So far, the sequences of over 300 vertebrate cadherins have been reported, and the virtually complete sets of cadherins encoded by the genomes of *Caenorhabditis elegans* and *Drosophila melanogaster* are now known.

Structural diversity of the cadherin superfamily

The recent explosion in genomic sequencing of various animals has shed new light on the diversity of the cadherin superfamily. In humans, more than 80 members of the cadherin superfamily have been sequenced. Current annotation of the *C. elegans* and the *Drosophila* genomes reveals 14 and 16 cadherin genes, respectively. Cadherins are defined by the presence of the cadherin domain (CD), a roughly 110 amino-acid peptide that mediates calcium-dependent homophilic interactions between cadherin molecules (FIG. 1). The CD is typically organized in tandem repeats. Calcium ions associate with the linker region that connects two CDs, and require interaction with amino acids from both CDs (FIG. 1; see below).

Here we present a classification of cadherins into

subfamilies on the basis of the domain layout of individual cadherins, which includes the number and sequence of CDs, and the presence of other conserved domains and sequence motifs (FIG. 2, TABLE 1). This analysis reveals that four cadherin subfamilies are conserved between *C. elegans*, *Drosophila* and humans: classic cadherins, Fat-like cadherins, seven-transmembrane cadherins and a new subfamily of cadherins that is related to *Drosophila* Cad102F. Classic cadherins break up in four subgroups, as listed in TABLE 1. Fat-like cadherins contain a subgroup of highly related molecules that we call Fatoid cadherins. These include all known vertebrate Fat-like cadherins, *Drosophila* Cad76E and *C. elegans* Cdh-4. Cadherins containing protein kinase domains are found in vertebrates (RET cadherins) and in *Drosophila*. Desmosomal cadherins are presumably derived from type I classic cadherins within the CHORDATE lineage, as neither desmosomal cadherins nor DESMOSOMES are found in *Drosophila* or *C. elegans*. Finally, protocadherins also seem to be limited to chordates. The grouping of cadherins into seven subfamilies, which is largely on the basis of the overall protein domain architecture, is corroborated by sequence comparison of CDs only (see online supplementary materials). Note that only about half of the cadherins found in *C. elegans* and *Drosophila* are part of identified subfamilies.

Cadherins are not found in yeast, and only a single, poorly conserved CD has been reported in a secreted protein from *Dictyostelium*[11], indicating that transmembrane proteins of the cadherin superfamily might have evolved to meet the need for the complex cell interactions that are required for the multicellular organization of METAZOANS. The function of classic cadherins during the formation and maintenance of epithelial tissues and cell–cell ADHERENS JUNCTIONS — two other metazoan inventions — is of particular importance. Within the classic cadherin subfamily, an interesting shift in protein organization has taken place during evolution — chordate classic cadherins lack non-chordate classic cadherin domains (NCCDs), laminin G (LG) and epidermal growth factor (EGF) domains and consistently contain five CDs, in contrast to the domain structure of classic cadherins in three other phyla: ECHINODERMS, ARTHROPODS and NEMATODES (FIG. 2, TABLE 1). This finding indicates that, during the early evolution of chordates, the structure of classic cadherins was modified and that a single progenitor might have given rise to the numerous classic cadherins found in vertebrates today, a conclusion supported by the phylogenetic analysis of chordate cadherins[12].

The cadherin families of *C. elegans* and *Drosophila* are small and very similar in size, and these cadherin genes are scattered throughout the genome without any obvious clustering, indicating that gene duplication events might not have occurred recently. So most of these genes were presumably established early during metazoan evolution. In particular, the progenitors of the four conserved subfamilies of cadherins are the result of a GENE RADIATION event that occurred before nematodes, arthropods and chordates diverged. In contrast, classic cadherins and the closely related desmosomal cadherins, as well as the more divergent protocadherins, were all amplified within the chordate lineage, resulting

CHORDATES
Phylum that comprises animals with a notochord and includes all vertebrates.

DESMOSOME
A patch-like adhesive intercellular junction found in vertebrate tissues that is linked to intermediate filaments.

METAZOANS
Refers to the kingdom Animalia (animals) that comprises roughly 35 phyla of multicellular organisms.

ADHERENS JUNCTIONS
Cell–cell or cell–matrix adhesive junctions that are linked to microfilaments.

ECHINODERMS
Animal phylum of marine invertebrates including sea urchins and starfish.

ARTHROPODS
Largest animal phylum composed of invertebrates that have a segmented body, segmented appendages and an external skeleton. This includes insects, spiders and crustaceans.

NEMATODES
Animal phylum of unsegmented roundworms.

GENE RADIATION
Process that leads to the formation of gene families in which gene amplification through gene duplication events is followed by the diversification of gene structure and function.

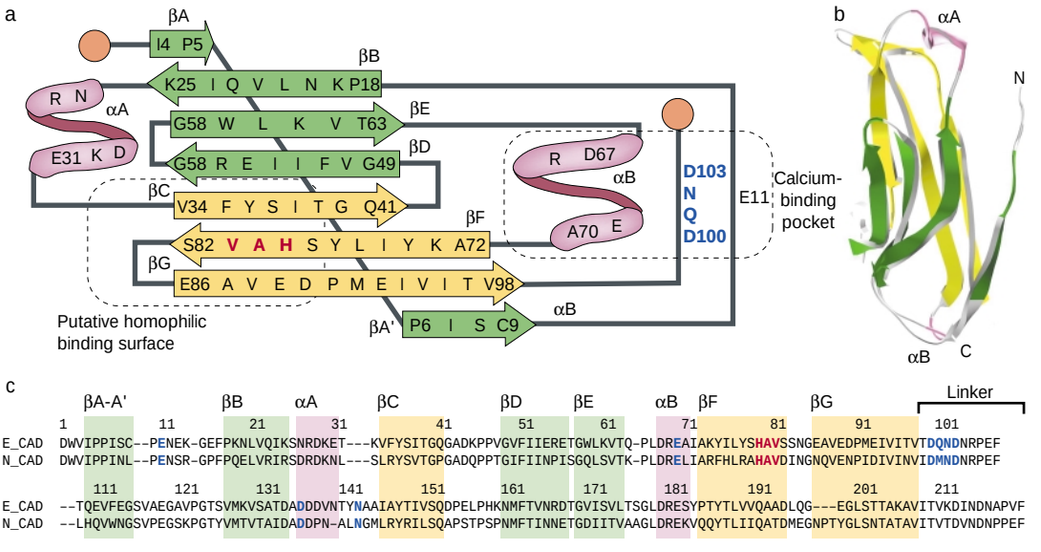

Figure 1 | **Structure of the cadherin domain.** The structure of the first cadherin domain (CD) of mouse E-cadherin is shown in **a** | and **b** |. It was solved by NMR spectroscopy[18] and, subsequently, the crystal structure of the first CD of N-cadherin revealed a similar topology[19]. The CD consists of a seven-strand β-sheet with the amino and carboxyl termini located at opposite ends of the molecule. The segment connecting strands B and C adopts an apparently helical structure consisting of a succession of β-turn and β-like hydrogen bonds. This unique quasi-β-helix structure is characteristic of the CD. **a** | Schematic of topology of the amino-terminal CD of mouse E-cadherin. βA, βA′, βB, βE, and βD (green) and βC, βF, and βG (yellow) form β-sheets. The α-helices are shown in magenta. The putative homophilic binding surface including the amino acids HAV (red), and the Ca^{2+}-binding pocket with the amino acids that interact with Ca^{2+} (blue), are indicated by the dotted lines. **b** | Ribbon structure of the CD. The colour coding is the same as in (**a**). **c** | Alignment of the first two N-terminal CDs of mouse E- and N-cadherin. The colour coding is the same as in (a). The CDs are connected by a roughly 10 amino-acid linker region. Note that the amino acids that form a single Ca^{2+} binding pocket (indicated in blue) are found in the first and second CDs and include animo acids in the linker region.

in numerous genes with pronounced clustering[13,14]. In humans, all six desmosomal cadherin genes are found in chromosomal region 18q12.1; three genes that encode more than 50 protocadherins are found in region 5q31–32, and many classic cadherins are organized in gene clusters[13–16]. A similar gene amplification is seen in other unrelated gene clusters, such as in the *C. elegans* collagen genes. It has been proposed that the

Table 1 | Cadherin subfamilies

Cadherin subfamily	Type	Cadherin	Species	No. of CDs
(I) Classic cadherins	***Vertebrate Type I classic cadherins***	Examples include:		
• Highly conserved cytoplasmic domain that binds to catenins	• HAV motif in first CD	E-cadherin (CDH1)	Hs	5
		P-cadherin (CDH3)	Hs	5
• Often found at adherens junctions		N-cadherin (CDH2)	Hs	5
		R-cadherin (CDH4)	Hs	5
	Vertebrate Type II classic cadherins	Examples include:		
	• No HAV motif in first CD	VE-cadherin (CDH5)	Hs	5
		Cadherin-7 (CDH7)	Hs	5
		Cadherin-8 (CDH8)	Hs	5
		Br-cadherin (CDH12)	Hs	5
	Ascidian classic cadherins	Cl-cadherin	Ci	5
		BS-cadherin	Bs	5
	Non-chordate classic cadherins	LvG-cadherin	Lv	17
	• Variable number of CDs	DE-cadherin	Dm	8
	• LG and EGF domains	DN-cadherin	Dm	18
	• NCCD domain, only found in these cadherins	Hmr-1 (splice products 1a/1b)	Ce	3/19*
(II) Fat-like cadherins	***Fatoid cadherins***	Examples include:		
• Very large extracellular domain with up to 34 CDs	• More than 30 CDs, closely related in sequence	hFat1	Hs	34
	• Flamingo box in some members	hFat2	Hs	34
• Heterogeneous subfamily	• LG and EGF domains	DCad76E (CG7749)	Dm	34
	• Conserved region in the cytoplasmic domain (between vertebrates and fly)	Cdh4 (F25F2.2)	Ce	32
	Other Fat-like cadherins	Fat	Dm	34
	• Variable number of CDs	Dachsous	Dm	27
	• Flamingo box in Fat	Cdh-3 (ZK112.7)	Ce	19
	• LG and EGF domains in Fat and Cdh-3	Cdh-1 (R10F2.2)	Ce	25
(III) Seven-pass transmembrane cadherins	• Seven-pass transmembrane domain similar to G-protein linked receptors (Secretins)	hFlamingo1	Hs	9
		hFlamingo2	Hs	9
	• Flamingo box	Flamingo/Starry night	Dm	9
	• LG and EGF domains	Cdh-6 (F15B9.7)	Ce	9
(IV) DCad102F-like cadherins	• Sequence conservation throughout much of the protein	KIAA0911	Hs	2
		KIAA0726	Hs	2
	• LG domain	DCad102F (CG11059)	Dm	2
	• Glu/Ser-rich cytoplasmic domain	Cdh-11 (B0034.3)	Ce	2
(V) Protein kinase cadherins	• Tyrosine kinase domain (RET-Cadherins)	RET	Hs	2
		DRet (CG1061+CG14396)	Dm	1‡
	• Putative Ser/Thr kinase	DCad96Ca (CG10244)	Dm	1
(VI) Desmosomal cadherins	***Desmocollins***	Desmocollin-1	Hs	5
• Only found in vertebrates	• Conserved cytoplasmic domain	Desmocollin-2	Hs	5
• Localize at desmosomes		Desmocollin-3	Hs	5
• Interact with plakoglobin, desmoplakin and plakophillins	***Desmogleins***	Desmoglein-1	Hs	5
	• Conserved cytoplasmic domain	Desmoglein-2	Hs	5
		Desmoglein-3	Hs	5
(VII) Protocadherins	***Protocadherins (Pcdh) α, β and γ***	Examples include:		
• Only found in vertebrates	• 52 protocadherins encoded by 3 genes	Pcdh-α3	Hs	6
	• all Pcdh-α/CNR proteins have a constant C-terminal cytoplasmic domain that interacts with Fyn tyrosine kinase	Pcdh-β1	Hs	6
		Pcdh-γA9	Hs	6
	Other protocadherins	Examples include:		
		Pcdh-1	Hs	6
		Pcdh-8 (Arcadlin)	Hs	6

*J. Petite, personal communication; ‡ R. Cagan, personal communication (Species: Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*, Ci, *Ciona intestinalis*; Bs, *Botryllus schlosseri*; Lv, *Lytechinus variegatus.* Domains: CD, cadherin domain; CNR, cadherin-related neuronal receptor; EGF, epidermal growth factor; LG, laminin G; NCCD, non-chordate classic cadherin domain.) Uncharacterized cadherins of *Drosophila* are named according to their cytological map position (for example, DCad 102F).

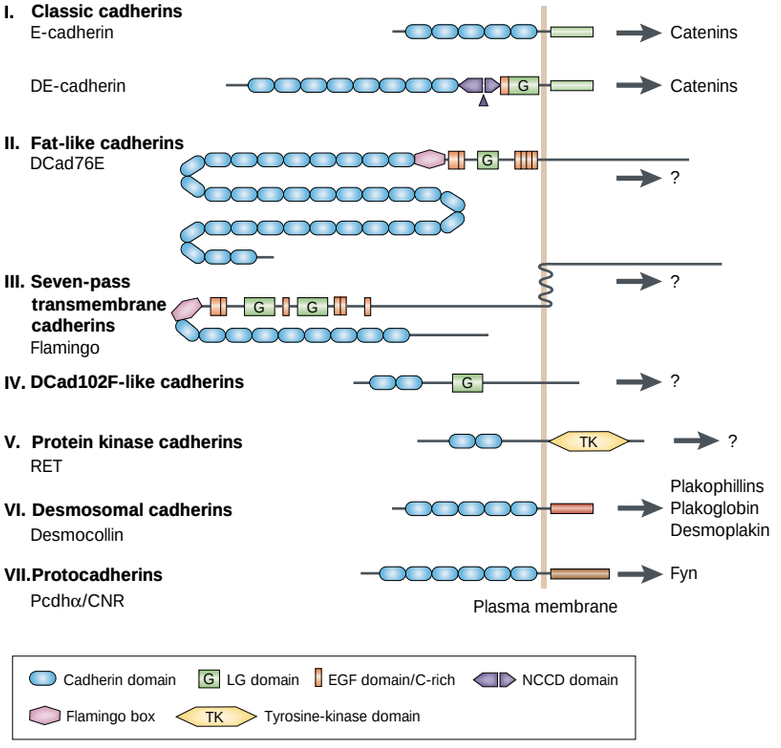

Figure 2 | **Structural diversity of the cadherin superfamily.** Representatives of each of the seven subfamilies of cadherins are shown. Subfamilies I to VII are conserved between nematodes (*Caernorhabditis elegans*), arthropods (*Drosophila melanogaster*) and chordates (humans). Members of subfamily V are found in chordates and *Drosophila*, whereas cadherins of subfamilies VI and VII are at present only known in vertebrates. Binding partners for the cytoplasmic tail of cadherins have been characterized for classic cadherins (subfamily I), for desmosomal cadherins (subfamily VI), and for Pcdhα/CNR protocadherins (subfamily VII). These interacting factors are listed at the right. It was recently shown that DE-cadherin is proteolytically cleaved within the NCCD domain during maturation (arrowhead)[95]. (NCCD, non-chordate classic cadherin domain; EGF, epidermal growth factor; LG, Laminin G)

formation of collagen gene clusters was caused by the evolution of a complex extracellular matrix, the nematode cuticle[17]. The amplification of cadherins in vertebrates might be explained by the more complex tissue interactions found in humans and other vertebrates compared with invertebrates, particularly the large increase in size and complexity in the central nervous system.

Structural basis of cell adhesion

Although it is broadly accepted that the predominant role of cadherins is to mediate adhesive interactions between cells, the mechanism of adhesive contact formation is still a matter of intense research. Structural studies have focused on vertebrate classic cadherins. These molecules are believed to form two types of dimers. Cadherins associate laterally within the same plasma membrane to form parallel *cis* dimers, and cadherins protruding from adjacent plasma membranes associate in an anti-parallel fashion to form *trans* dimers. The structure of the first CD of E-cadherin[18] and of N-cadherin[19] revealed that the cadherin fold consists of a seven-strand β-sheet with its amino and carboxyl termini located at opposite ends of the molecule (FIG. 1b). The crystal structures of peptides containing the first and second CDs (CD1 and CD2) of E-cadherin[20,21], or of N-cadherin[22], indicate that calcium is central in *cis*-dimer formation. Each dimer associates with six calcium ions through residues that are located in the linker region between CD1 and CD2 (FIG. 3a). Single amino-acid substitutions in the calcium binding sites can disrupt cell aggregation *in vivo*[23]. Calcium binding makes CDs arrange in a rigid structure [20,21,24,25] that is resistant to proteolysis[26].

Crystallographic analysis on the first and second CDs of E-cadherin and N-cadherin have provided clues as to how cadherin molecules induce lateral clustering essential for the formation of a stable adhesive interface between adjacent cells. Although different mechanisms underlying *cis* dimerization have been observed in the crystal structures of different cadherin molecules[19,20,22], an emerging theme is that two cadherin molecules form a *cis* dimer that functions as a building block for lateral clustering. These and other studies indicate that *cis* dimerization or more extensive lateral clustering is a prerequisite for stable cell adhesion[27–29]. Although *cis* dimers might primarily form as homodimers, the formation of functional *cis* heterodimers between N- and R-cadherin has been reported[30].

Adhesion between opposing cell membranes requires the formation of *trans* dimers (FIG. 3b). The mechanism of *trans*-dimer formation is, at present, controversial. Several studies indicate that *trans* dimers form by interactions between the amino-terminal CDs of opposing cadherin molecules[19,21,28,31]. These data are corroborated by early findings that located the homophilic binding specificity of classic cadherins within the amino-terminal CD[32,33]. On the basis of the crystal structure of the first CD from N-cadherin, a zipper model for *trans* dimerization was proposed that involved only the tip of the amino-terminal CD[19]. This model provided an early foundation for understanding the mechanics of the cell adhesion interface. However, the subsequent crystal structures of CD1 and CD2 from N-cadherin[22] and E-cadherin[20,21] did not show the adhesion interface seen in the first CD of N-cadherin. In addition, a recent biophysical study indicates a different type of *trans*-dimer association, in which the five CDs show variable degrees of lateral overlap, including the complete anti-parallel overlap of all five CDs (FIG. 3b)[34]. In the presence of calcium, the extracellular part of vertebrate classic cadherins forms a rod-like structure of about 20 nm in length with each individual CD spanning about 4.5 nm (REFS 19,20,25). Full lateral overlap of *trans* dimers would imply a distance between adjacent plasma membranes of 20–25 nm, a value consistent with the distance between plasma membranes at adherens junctions that is found in ultrastructural studies.

Adhesive contacts and adherens junctions

Two types of adhesive contacts are mediated by classic cadherins: diffuse adhesive contacts all along a cell–cell contact surface, and more discrete contacts by ultrastructurally defined adherens junctions, such as the

ZONULA ADHERENS
A cell–cell adherens junction that forms a circumferential belt around the apical pole of epithelial cells.

PDZ DOMAINS
Protein–protein interaction domain, first found in PSD-95, DLG and ZO-1.

SH3 DOMAINS
Src homology region 3 domains. Protein sequences of about 50 amino acids that recognize and bind sequences rich in proline.

FILOPODIUM
Finger-like exploratory cell extension found in crawling cells and growth cones.

LAMELLIPODIUM
Thin sheet-like cell extension found at the leading edge of crawling cells or growth cones

ZONULA ADHERENS. Diffuse adhesive contacts probably involve the oligomerization of cadherin *trans* dimers, as individual *trans* dimers provide little adhesive strength[25,29]. Adherens junctions could simply represent very large arrays of *trans* dimers. However, the situation seems to be more elaborate, as cadherins might not be the principal components of adherens junctions, at least in some cases. Indeed, adherens junctions can form in the absence of Hmr-1 cadherin, the only classic cadherin in *C. elegans*, or in the absence of its associated catenins[35,36]. In mouse and *Drosophila* embryos, where E-cadherin or DE-cadherin, respectively, are essential for adherens junction assembly and epithelial integrity, markedly reduced levels of these cadherins can still support the formation of normally sized adherens junctions[37–39]. These observations are inconsistent with a model in which adherens junctions simply represent a large array of cadherins and associated cytoplasmic proteins. Instead, cadherin *trans* dimers probably form small clusters separated by other proteins, and the density of these clusters in the adherens junctions may vary considerably without affecting the size of the adherens junction. A novel protein complex has recently been characterized that is concentrated at adherens junctions[40–42]. This complex is composed of Nectin, a transmembrane protein of the immunoglobulin superfamily that interacts with the PDZ-DOMAIN protein Afadin, which in turn can bind to Ponsin, a protein containing three SH3 DOMAINS (FIG. 4a). This complex can interact with the actin cytoskeleton. Nectin and cadherin complexes interact with each other and are recruited together to adherens junctions[43]. Initial functional studies indicate that Afadin is important for junctional organization and epithelial integrity[44]. So adherens junctions contain two complexes that interact with each other and with the actin cytoskeleton.

Cell and tissue polarity

Epithelial cells provide a clear example of cell polarity, with various molecules, including proteins, sorting to distinct apical and basolateral membrane domains. The crucial role of classic cadherins and their associated catenins in epithelial differentiation has been well documented, and these protein complexes seem to be broadly important for forming and maintaining epithelial tissues[45,46]. Conversely, downregulation of classic cadherins, such as E-cadherin or DE-cadherin, is often associated with a loss of epithelial morphology during normal development and in many carcinomas. The zinc-finger transcription factor Snail is important for repressing the expression of DE-cadherin and E-cadherin in non-epithelial cells[47–49].

Epithelial cells usually form a continuous tissue structure. However, at certain times during normal development, or in experimental cell-culture models, epithelial cells have free edges that approach each other to establish new lateral contacts[36,50,51]. The initial contact between cells is made by FILOPODIA or LAMELLIPODIA, and such contacts are stabilized by classic cadherins. When these contacts broaden, cadherins concentrate in discrete puncta. The adhesive interactions are further stabilized through linkage of cadherins to the cytoskeleton and, eventually, by the formation of mature adherens junctions. Cadherin-mediated adhesion leads to

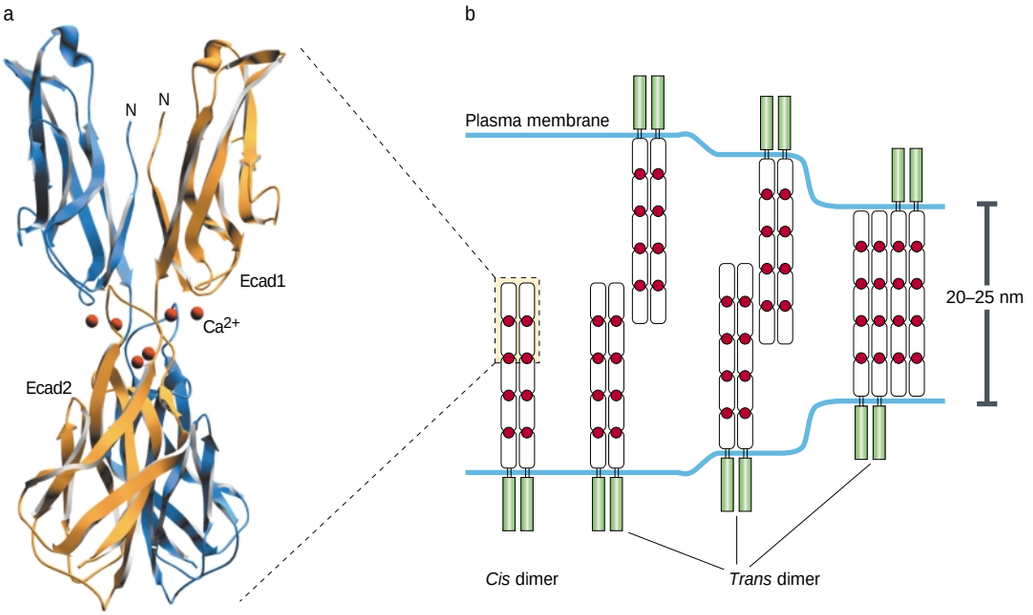

Figure 3 | **Ca^{2+}-mediated *cis*- and *trans*-dimer formation of vertebrate classic cadherins. a** | Dimer interface between two N-terminal repeats of E-cadherin domains 1 (Ecad1) and 2 (Ecad2). Each cadherin molecule binds three calcium ions that are important in the rigidification and *cis*-dimer association of cadherins[20,21]. **b** | A *cis* dimer consists of two cadherin molecules within the same plasma membrane that are associated laterally. The pairs of cadherin molecules from opposing cells that associate with one another are referred to as *trans* dimers. Different models for *trans*-dimer formation have been proposed that suggest different extents of lateral overlap between the extracellular regions. Red dots indicate the location of Ca^{2+} ions between adjacent CDs.

FOLLICLE CELLS
In this review, the term follicle cells refers to cells that surround the developing insect egg and secrete the egg membranes, the chorion and vitelline envelope.

GASTRULATION
Series of morphogenetic movements observed during the early development of most animals that leads to the formation of a multilayered embryo with an outer cell layer (ectoderm), an inner cell layer (endoderm), and an intermediate cell layer (mesoderm).

recruitment of specific cytoskeletal factors, such as the actin-associated factor Mena[51] and other transmembrane proteins, to cell–cell contact sites[46].

Cadherins seem to be directly involved in maintaining cell polarity by directing the localization of the sec6/8 complex, which specifies vesicle targeting to the lateral membrane[52]. This recruitment, and the continuous polarized delivery of specific molecular components to the lateral membrane, establishes and maintains the lateral membrane domain of epithelial cells and contributes to epithelial apical–basal polarity[46,52]. Interestingly, in fully polarized epithelial cells, the sec6/8 complex is not found along the entire lateral membrane but is concentrated in close association with apical adherens junctions, indicating a potentially direct molecular link between the cadherin–catenin complex and the vesicle targeting machinery (FIG. 4a)[52].

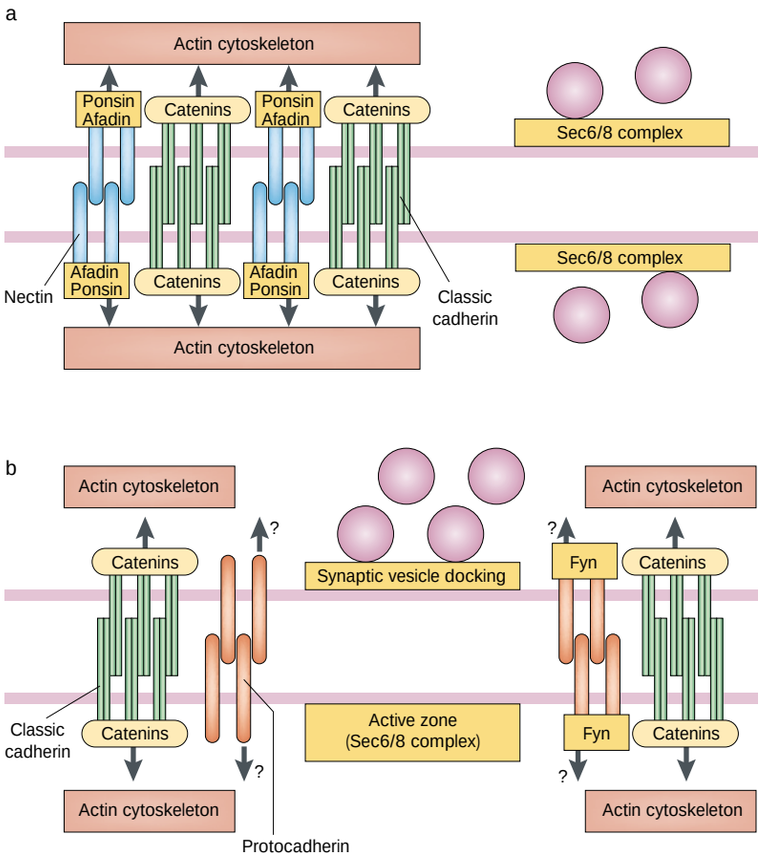

Figure 4 | **Comparison between cadherin-mediated adhesive interactions at epithelial and synaptic adherens junctions. a** | Schematic of the zonula adherens, a circumferential adherens junction found in epithelial cells. This junction contains the classic cadherin–catenin complex and the recently identified nectin/afadin/ponsin complex[40–42]. Both complexes interact with the actin cytoskeleton and with each other[10,43]. The zonula adherens is closely associated with a vesicle docking site that contains the Sec6/8 complex[52]. **b** | At the interneuronal synapse, we also find a close association between adherens junctions and vesicle docking zones. The sec6/8 complex was found to associate with the postsynaptic membrane only during synaptogenesis[96]. The classic cadherin–catenin complex is a principal component of synaptic adherens junctions similar to the zonula adherens. Protocadherins that localize to synapses include Arcadlin[84] and the Pcdhα/CNR protocadherins[75]. Whether protocadherins contribute to synaptic adhesion remains to be established. The Pcdhα/CNR protocadherins interact with the cytoplasmic protein kinase Fyn, and seem to reside within the active zone, indicating that they might have a primary role in signalling rather than adhesion[13,75].

In addition to their role in apical–basal polarity, cadherin superfamily members were recently implicated in a second form of cell polarity called planar epithelial polarity (FIG. 5). This property is found in many epithelia. One example is the fly wing epithelium, where each cell polarizes its actin cytoskeleton along the proximal–distal axis, such that a bundle of actin filaments polymerizes and projects from the surface at the distal-most vertex of each cell, ultimately forming a wing hair (FIG. 5a). Genes involved in establishing the planar polarity of the wing epithelium include components of the Wnt/Frizzled signalling pathway, Frizzled and Dishevelled[53], and three different cadherins, Fat, Dachsous[54] and Flamingo/Starry Night[55,56]. Although the mechanism by which cadherins affect planar polarity is unknown, it was found that Flamingo/Starry Night adopts an asymmetric distribution during polarity establishment, becoming enriched at the proximal and distal cell surfaces (FIG. 5b)[55]. Planar polarity also influences polymerization of the microtubule cytoskeleton, manifesting itself through orientation of the mitotic spindle and thereby the axis of cell division along the body axes. Studies in both *C. elegans* and *Drosophila* implicate the Wnt/Frizzled pathway in this process[53]. Many planar polarity genes have not been examined in this context, but it is at least clear that Flamingo/Starry Night is essential for spindle orientation[57].

Two other examples that highlight important roles of cadherins in generating asymmetric tissue organization are the contribution of DE-cadherin to the formation of the anterior–posterior axis in *Drosophila*, and the function of N-cadherin in setting up left–right asymmetry in the chick. A cell sorting process that is driven by different levels of DE-cadherin directs the oocyte to the posterior pole of the egg chamber during *Drosophila* oogenesis[8,58]. This highly reproducible positioning event allows the oocyte to interact with a specific group of FOLLICLE CELLS, thereby initiating a cascade of cell interactions that are crucial for the formation of the embryonic anterior–posterior axis. Disruption of the function of N-cadherin during chick GASTRULATION leads to a random orientation of the heart along the left–right axis[59]. Asymmetric N-cadherin expression and cell movements that prefigure the position of the heart and other organs along the left–right axis are seen during gastrulation. How N-cadherin contributes to these asymmetric cell movements remains a mystery.

Cell movement

Many of the changes in cell shape or movement observed during development occur while cells are in direct contact and require, therefore, dynamic changes in adhesive interactions. These changes may play a permissive role, as the release of adhesion is important for the relative movement of cells that are in contact. However, adhesive interactions also directly promote movement, as traction must be generated between cells for cell rearrangements to occur in solid tissues. To determine whether changes in cadherin activity play a permissive or a more active role can be difficult, as illustrated by the analysis of C-cadherin function during

NEUROEPITHELIUM
Epithelial layer of cells that gives rise to the nervous system.

NEURULATION
Morphogenetic process during which the progenitors of the nervous system segregate from the ectoderm.

convergent extension movements in *Xenopus* gastrulation. In this process, cells move towards the dorsal midline of the embryo, thereby rearranging by cell intercalation, leading to an extension of the embryo along the anterior–posterior axis (FIG. 6a)[60]. Adhesion mediated by the classic cadherin C-cadherin must be reduced to permit these movements to occur[61]. However, the disruption of C-cadherin activity causes defects not only during gastrulation movements, but also in tissue structure[62], raising the possibility that the disruptions in cell movement might be a secondary consequence of a compromised cell architecture. Similar difficulties have emerged from the analysis of DE-cadherin in embryonic morphogenesis where its role in epithelial maintenance might mask a function in promoting cell rearrangements[37,38].

Paraxial protocadherin (PAPC) seems to be directly involved in convergent extension in *Xenopus* and zebrafish embryos, where it is expressed in the mesoderm during gastrulation[63,64]. PAPC, which can promote homotypic cell adhesion, is required for convergent extension of the mesoderm. Notably, overexpression of PAPC can promote convergent extension under certain experimental conditions[63]. These findings argue that PAPC acts as an adhesion receptor that directly promotes cell movement, perhaps providing traction needed for cell motility. Alternative and non-exclusive possibilities are that PAPC is primarily a signalling receptor, as was suggested for other protocadherins[13]. PAPC activity might also generate the tissue polarization that is observed during convergent extension (FIG. 6a)[60,63], functioning similarly to the activity of other cadherins in planar epithelial polarization, outlined above. Intriguingly, cell polarization during convergent extension resembles planar polarity in that it also requires Wnt/Frizzled signalling[65–67].

The requirement for DE-cadherin in cell migration during *Drosophila* oogenesis is a convincing example for a direct role of classic cadherins in cell migration on a cellular substrate. DE-cadherin is involved in the migration of a small group of somatic cells, the 'border' cells, on the surface of the much larger germline cells[68] (FIG. 6b). It is required in both the somatic and germline cells for this movement to occur. DE-cadherin is not required for the formation of the border cell cluster and, more importantly, is not required for maintaining integrity of the border cell cluster during migration. In the case of integrin-based cell migration, it was shown that intermediate levels of adhesion to the substrate promotes maximal migration speed, with both positive and negative deviations slowing or halting motility[69]. Similarly, reduction in the level of DE-cadherin reduces the speed of border cell migration[68], indicating that DE-cadherin might not have just a permissive role, but might be the key adhesion molecule that provides traction for border cells to travel over germline cells.

Organization of the nervous system

Various cadherins are expressed in the nervous system in complex patterns. The first example was N-cadherin, which is broadly expressed in the NEUROEPITHELIUM, beginning at NEURULATION. This expression pattern led to the speculation that N-cadherin might be critical for the segregation of neural and epidermal tissues during neural tube formation. However, an essential role in neurulation was disproved by the knockout of mouse *N-cadherin*[70]. After neurulation, but before neuronal differentiation, many classic cadherins, including N-cadherin, are expressed in the developing central nervous system (CNS) in a region-specific manner that often coincides with morphological boundaries[71]. The most direct evidence that these cadherins contribute to the subdivision of the neuroepithelium has come from analysis of the *Xenopus* type II classic cadherin F-cadherin[72]. The expression of F-cadherin confines neuroepithelial cells to the sulcus limitans, a region separating the dorsal and ventral halves of the caudal neural tube. One apparent consequence of F-cadherin expression in

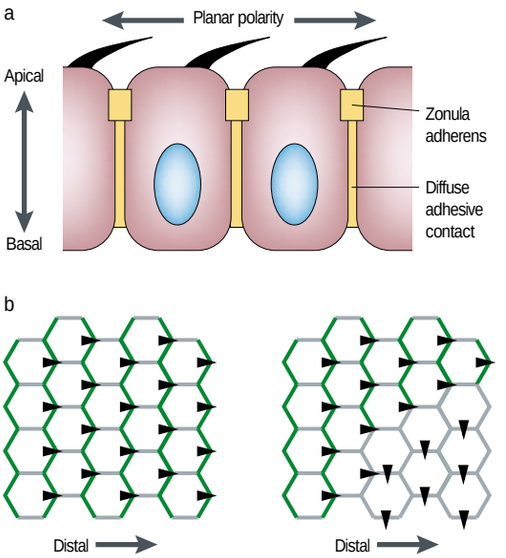

Figure 5 | **Cadherins in apicobasal and planar epithelial polarity. a** | The example depicted here is the wing epithelium of *Drosophila melanogaster,* shown in cross section. Classic cadherins mediate lateral cell contacts (yellow) between epithelial cells that can take the form of either a diffuse adhesive contact, or an adherens junction, such as the zonula adherens, that can be seen in electron micrographs as an electron-dense specialization of the plasma membrane. Epithelial sheets are obviously different across their apical–basal axis, but many epithelial cells can also discern directions in the plane of the epithelium with respect to the organ or body axis of which they are a part. They use this information to polarize their actin and microtubule cytoskeletons along this axis. The most obvious indication of planar polarity in the wing epithelium is the hair that is formed by each cell. Hairs emerge from a distal region of the apical cell surface and all point distally. **b** | The array of cells in a *Drosophila* wing epithelium, viewed from above. A wild-type array is shown on the left, illustrating the uniform planar polarity (wing hair orientation) and the distribution of Flamingo/Starry Night (green), which accumulates at the proximal and distal surfaces of every cell. The right array shows a group of cells that contain Flamingo/Starry Night mutant cells (absence of green), in which the orientation of wing hairs and therefore the axis of planar polarity has shifted[55,56].

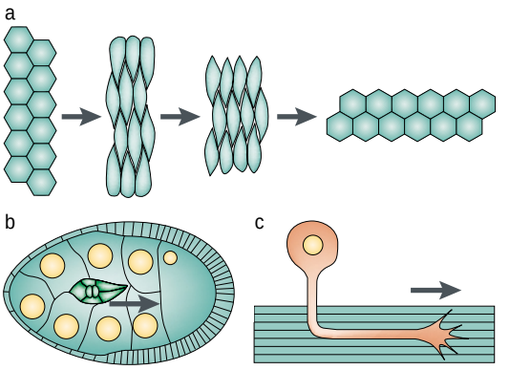

Figure 6 | **Cell movements that involve cadherins. a** | Convergent extension is a cell rearrangement that involves the transient polarization of cells, which then move, converging towards the centre of the tissue. Cell intercalation leads to an extension of the tissue perpendicular to the axis of convergence. This movement is seen, for example, during frog gastrulation, where it may involve C-cadherin and paraxial protocadherin[61,63]. **b** | The border cell cluster (green) is a small group of *Drosophila* follicle cells that migrate on the surface of much larger germline cells (green/yellow). This movement is driven by DE-cadherin, which is required in both border cells and germline cells[68]. **c** | N-cadherin and DN-cadherin mediate the movement of neuronal growth cones (red) on cellular substrates such as axon bundles (green)[76–78].

the sulcus limitans is that these cells remain a coherent group and do not participate in the extensive cell rearrangments that take place during neurulation.

Protocadherins also contribute to CNS regionalization by controlling the migration of neurons that will organize into different cortical layers during brain morphogenesis. Although cadherins, including protocadherins, are generally viewed as homophilic adhesion molecules, recent work indicates that the Pcdhα/CNR protocadherins might also function as receptors or co-receptors for extracellular ligands in the brain[13,73]. This work began with studies of the secreted molecule Reelin, identified because mutant mice have a marked behavioural disorder. Two protein families have been shown to function as reelin receptors, perhaps as a heteromeric complex: members of the LDL-receptor-related protein family, which couple to the cytoplasmic adaptor protein mDab1; and members of the Pcdhα/CNR family, which bind the non-receptor tyrosine kinase Fyn[73,74]. As Pcdhα/CNR protocadherins show considerable molecular diversity and differential expression patterns within local brain areas, it is possible that Reelin receptor complexes that contain different Pcdhα/CNR protocadherins are instructive in positioning and differentiating neuronal sub-populations within the cortex[73,75].

Classic cadherins are also important during the outgrowth of NEURITES and during axonal patterning and FASCICULATION (FIG. 6c). Early studies that indicated that N-cadherin can function as a substrate for neurite extension in cultured cells were reinforced by the finding that N-cadherin is required for the normal outgrowth and guidance of retinal axons[76,77]. In *Drosophila*, DN-cadherin is the only classic cadherin expressed in the developing embryonic CNS[78]. Null mutations in *DN-cadherin* and the *Drosophila* catenin gene *armadillo* affect axon outgrowth, although in a mild fashion, with many axons finding their targets appropriately[78,79]. In this respect, cadherins resemble various other axon guidance cues that direct axon outgrowth in a combinatorial fashion, with individual cues having subtle functions[9]. In addition, it was recently found that the patterning of dendrites that extend from *Drosophila* sensory neurons requires Flamingo/Starry Night[80].

Both classic and protocadherins localize to synapses, indicating that they may contribute to the generation of adhesive specificity needed to build complex neural networks[75,81–84]. The synapse is an adhesive contact between two neurons, with the transmitter release zone framed by adherens junctions that are ultrastructurally similar to epithelial adherens junctions, and that share with them the cadherin–catenin complex as a principal molecular component (FIG. 4b)[82,83]. In synapses, as in epithelial cells, adherens junctions are closely associated with vesicle-release zones (FIG. 4). It is believed that classic cadherins are important during synaptic adhesion, whereas the adhesive role of protocadherins at synapses remains to be clarified. Recent intriguing evidence indicates that synaptic activity can change the distribution and adhesive state of N-cadherin[85]. Cadherins, in turn, can influence synaptic activity[84,86,87]. These findings indicate an intimate relationship between synaptic adhesion and activity, raising the possibility that cadherins are important regulators of synaptic plasticity and activity modulation[13,89].

Some classic cadherins, such as cadherin-6, are expressed in groups of neurons that form neural circuits, indicating that cadherins might functionally integrate such neural circuits[71,90]. Protocadherins are also expressed in divergent and restricted patterns in the CNS, indicating that they might have a function in integrating neural circuits[91,92]. Moreover, the protocadherin genes *Pcdhα*, *Pcdhβ* and *Pcdhγ* can give rise to over 50 protocadherins[18] and the Pcdhα/CNR proteins have a differential expression pattern within individual brain areas[75]. These findings raise the possibility that a 'cadherin code' exists, which could identify individual neurons and their synaptic contacts[75,89], although neurexins and immunoglobulin-type adhesion receptors have also been proposed to contribute to synaptic specificity[93,94]. The structures of the *Pcdhα*, *Pcdhβ* and *Pcdhγ* genes show provocative similarities to the gene organization of immunoglobulins or T-cell receptors, which has led to the proposal that gene rearrangement might function in determining protocadherin expression patterns[13,15,16], a speculation that remains to be proved.

The future

The analysis of cadherins emphasizes the similarities between embryonic and neural morphogenesis. Cadherins have emerged as the predominant group of cell–cell adhesion molecules involved in embryonic morphogenesis, determining cell and tissue architecture, and controlling dynamic changes in cell shape and position. The role of individual cadherins in several specific morphogenetic processes has been determined, which

NEURITE
Process extended by a nerve cell that can give rise to an axon or a dendrite.

FASCICULATION
Bundling of axonal processes of neurons.

will now allow the study of how the adhesive activity of cadherins is modulated by cell signalling to facilitate coordinated cell behaviour. Cadherins are also important during neural morphogenesis, although the functional significance of cadherins in neural development remains less well understood. One important challenge will be to determine the exact mechanism underlying the specificity and stability of cadherin-mediated cell–cell adhesion, and to explore the variation in adhesive mechanisms and cellular responses of different types of cadherins. A second important challenge will be to substantiate the conjecture that cadherins provide an adhesive code that controls synaptic specificity. Elucidating whether and how different families of adhesion receptors cooperate in this process will represent an enormous advance in our understanding of complex neural network formation.

Links

DATABASE INFORMATION | Cadherin domain | Cadherins | RET | Protocadherins | EGF-like domain | LG domain | E-cadherin | N-cadherin | R-cadherin | Catenins | DE-cadherin | Nectin | Afadin | Ponsin | Snail | Mena | Frizzled | Disheveled | Fat | Dachsous | Flamingo/ Starry night | Reelin | LDL-receptor | mDab1 | Fyn | DN-Cadherin | Armadillo | Cadherin-6 | Pcdhα | Pcdhβ | Pcdhγ | Neurexin

FURTHER INFORMATION The cadherin resource | Godt and Tepass labs *Drosophila* cadherin resource | Cadherin web site at the LMB, Cambridge | Pfeifer lab page | Ikura lab page | Godt lab page | Tepass lab page

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