The cadherin superfamily: diversity in form and function

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Summary

Over recent years cadherins have emerged as a growing superfamily of molecules, and a complex picture of their structure and their biological functions is becoming apparent. Variation in their extracellular region leads to the large potential for recognition properties of this superfamily. This is demonstrated strikingly by the recently discovered FYN-binding CNR-protocadherins; these exhibit alternative expression of the extracellular portion, which could lead to distinct cell recognition in different neuronal populations, whereas their cytoplasmic part, and therefore intracellular interactions, is constant. Diversity in the cytoplasmic moiety of the cadherins imparts specificity to their interactions with cytoplasmic components; for example, classical cadherins interact with catenins and the actin filament network, desmosomal cadherins interact with catenins and the intermediate filament system and CNR-cadherins interact with the SRC-family kinase FYN. Recent evidence suggests that CNR-cadherins, 7TM-cadherins and T-cadherin, which is tethered to the membrane by a GPI anchor, all localise to lipid rafts, specialised cell membrane domains rich in signalling molecules. Originally thought of as cell adhesion molecules, cadherin superfamily molecules are now known to be involved in many biological processes, such as cell recognition, cell signalling, cell communication, morphogenesis, angiogenesis and possibly even neurotransmission.

Key words: Cadherin, Cell adhesion, Catenin, Signaling, Lipid raft

Introduction

In the early 1980s Jacob and co-workers first described E-cadherin (uvomorulin), a cell surface glycoprotein involved in compaction (Hyafil et al., 1981; Peyrieras et al., 1983), and the mouse gene was later cloned (Schuh et al., 1986). Subsequently, a calcium-dependent transmembrane cell-cell adhesion protein with prominent expression in neural tissue, N-cadherin, was cloned and analysed (Nose et al., 1987). This molecule shared common amino acid sequences throughout its entire sequence with chicken L-CAM/E-cadherin and mouse uvomorulin/E-cadherin as well as the then recently cloned placental P-cadherin. These proteins are now all known to be part of the classical cadherin family, members of which confer calcium-dependent intercellular adhesion.

Since then, the discovery of novel cadherins has exploded, and new ones are regularly reported. It is now clear that the classical cadherins are only a fraction of the cadherin-related molecules and that these constitute a cadherin superfamily that has a multitude of diverse members (see Fig. 1). Their extracellular portions, although containing conserved calcium-binding domains, show a plethora of divergent structural arrangements. They can have 5 to 34 definitive calcium-binding domain repeats of ~110 residues. The anchor to the cell membrane is usually a transmembrane region but can, as in the case of T-cadherin, be a glycosylphosphatidylinositol (GPI) anchor. A diverse array of cytoplasmic regions allows them specific interactions with their respective intracellular binding partners. Their spatial and temporal expression is complex and highly specific to the particular cell that synthesises them, even within a single tissue. So far, cadherins have been shown to be involved in many biological processes including cell adhesion, morphogenesis, cytoskeletal organisation and cell sorting/migration, as well as in pathological conditions such as cancer (Christofori and Semb, 1999). Here, we focus on the emerging diversity of structure and function of members of this large protein family, highlighting novel functions of cadherins rather than attempting to be exhaustive.

Structure-function relationships of cadherins

The classical cadherins are single-span transmembrane proteins located primarily within adherens junctions, which confer calcium-dependent cell-cell adhesion. They can transfer information intracellularly by interacting with a complex network of cytoskeletal and signalling molecules. Classical cadherins are modular proteins, mediating calcium-dependent cell-cell adhesion through their five extracellular calcium-binding repeats (Fig. 2A). E-cadherin and N-cadherin have been best characterised and studied; it has long been known that expression of either on the cell surface leads to cell sorting, homophilic interaction specificity being conferred by their specific extracellular regions (Yap et al., 1997b). Their intracellular regions link them with their cytoplasmic partners β-catenin or plakoglobin (PG) and consequently to α-catenin and the actin filament network (Yap et al., 1997a).

In recent years considerable light has been shed on the molecular mechanism of cell adhesion by classical cadherins from both structural and functional studies. We cover these briefly here but refer readers to some excellent reviews for

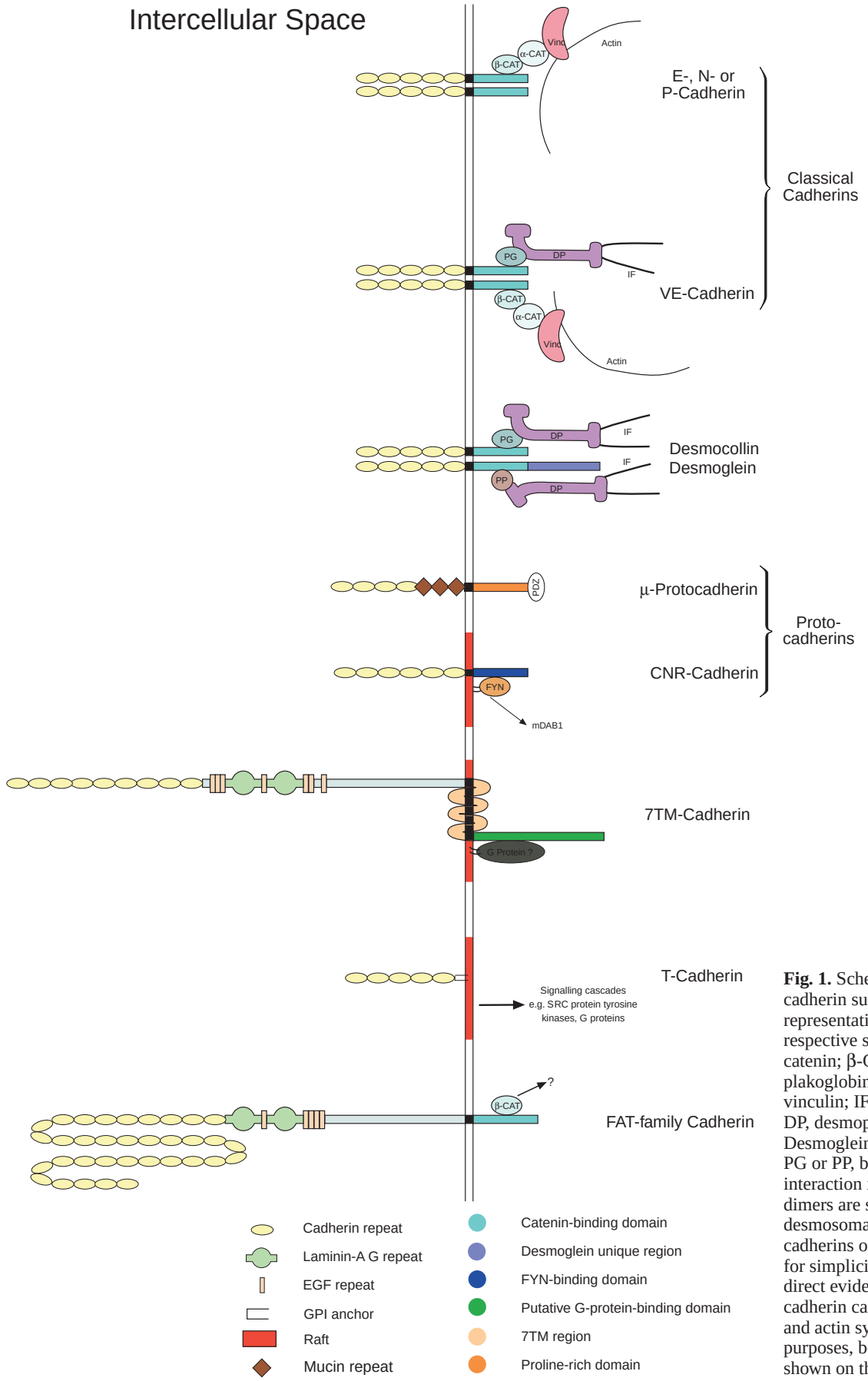

Fig. 1. Schematic overview of the cadherin superfamily depicting representative molecules for the respective subfamilies. α-CAT, α-catenin; β-CAT, β-catenin; PG, plakoglobin; PP, plakophilin; Vinc, vinculin; IF, intermediate filament; DP, desmoplakin. Desmocollin and Desmoglein can each bind to DP via PG or PP, but, for simplicity, only one interaction is illustrated. Lateral dimers are shown for classical and desmosomal cadherins. For the other cadherins only monomers are shown – for simplicity and because of lack of direct evidence for dimers. VE-cadherin can interact with both the IF and actin systems; for illustrative purposes, both types of interaction are shown on the same lateral dimer.

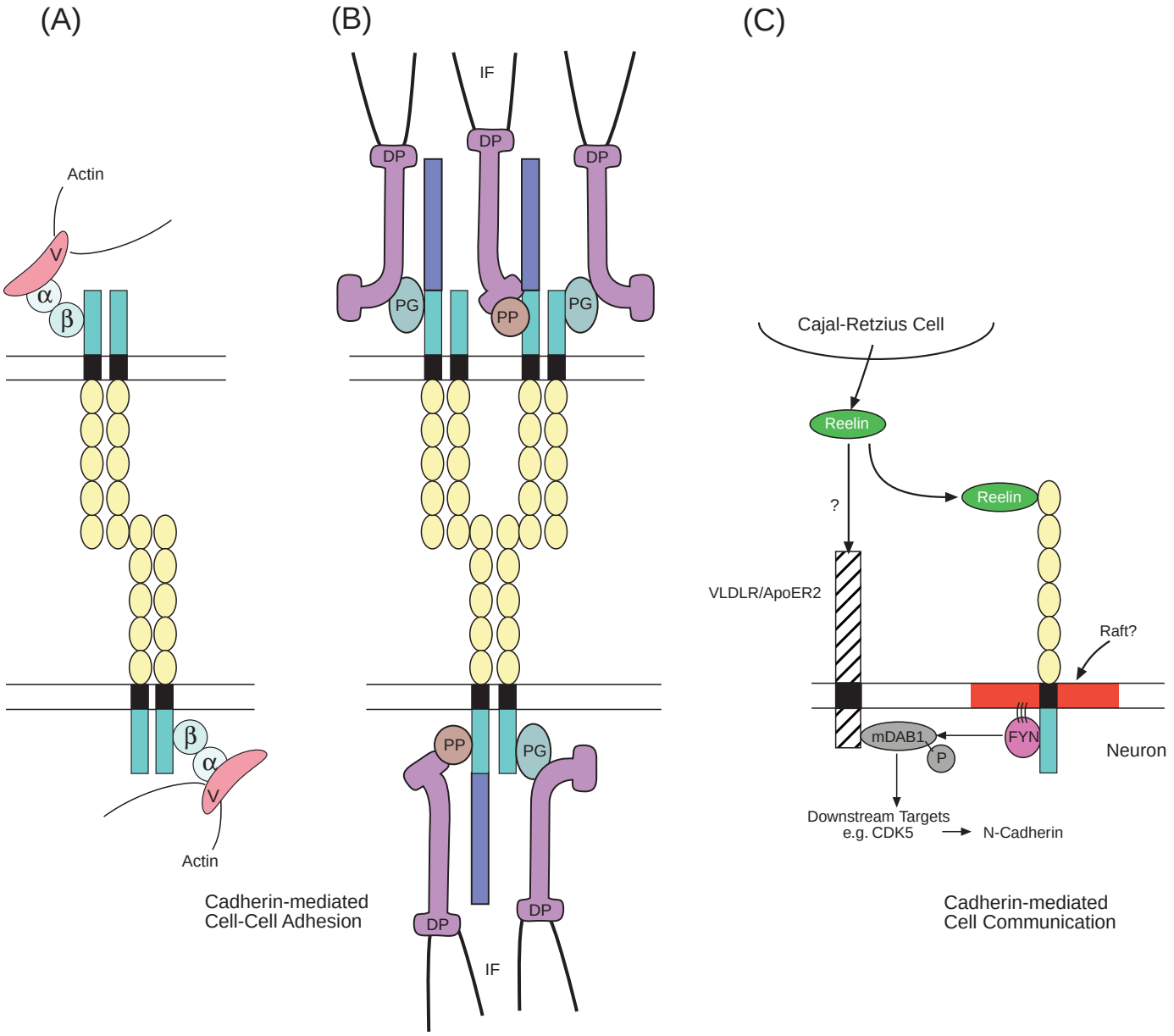

Fig. 2. Cadherin-mediated biological functions: (A) Classical cadherin-mediated cell-cell adhesion: classical cadherins form lateral homodimers, which interact with homodimers of the neighbouring cell through cadherin repeat 1. Intracellularly they confer adhesion by binding to their cytoplasmic partners, e.g. α-catenin (α), β-catenin (β), vinculin (v), and to the actin filament network. (B) Desmosomal cadherin-mediated cell-cell adhesion: desmosomal cadherins form lateral dimers, probably heterophilically, and interact with dimers on the neighbouring cell, probably via DSC-DSG interaction. Intracellularly they interact with the constituents of the desmosomal plaque (plakoglobin (PG), plakophilin (PP), desmoplakin (DP)) and the intermediate filament network (IF). (C) Cadherin-based cell communication: in the nervous system, the Cajal-Retzius cells secrete Reelin, which binds to specific neurons carrying CNR-cadherins on their cell surface. The SRC family tyrosine kinase FYN, bound to CNR-cadherin, tyrosine phosphorylates (P) mDAB1, which can, for example, activate CDK5 and several of its downstream targets. In N-cadherin-positive neurons, this pathway via CDK5 can lead to a reduction in N-cadherin-mediated adhesion. FYN associates via its attached fatty acids with lipid rafts, which may therefore be involved in signaling from CNR-cadherins. Lateral and head-to-head dimers are shown for classical (A) and desmosomal (B) cadherins; for CNR-cadherins only the monomer is shown.

more detail (Koch et al., 1999; Yap et al., 1997a). Several structural studies of single or double extracellular cadherin repeats give a consistent picture of the fold of an individual cadherin repeat, which bears a striking resemblance to the immunoglobulin fold despite little sequence homology. However, it is in the interactions between cadherin domains where much controversy has emerged. Examination of the crystal packing in the earliest X-ray structure of a single N-cadherin N-terminal domain (Shapiro et al., 1995) led the authors to propose that the cadherin dimerises laterally at the cell surface and also makes head-to-head contacts across the intercellular gap to form a so-called adhesive zipper (Fig. 2A). A key feature of this model is the exchange of β-sheets between partners in the lateral dimer and the cross-insertion of Trp2, an

essential residue for adhesive function, into a hydrophobic pocket in the apposed partner.

Although these ideas still hold true in general terms, the details of the interactions are probably substantially different from those originally conceived. Functional studies strongly support a role for dimerisation in adhesive function (Brieher et al., 1996; Takeda et al., 1999). There is also a great deal of evidence for lateral dimerisation from crystal structures of two-domain E-cadherin and N-cadherin constructs (Tamura et al., 1998; Pertz et al., 1999), but the contacts seen in the one-domain structure were probably artifacts of crystal packing that were due to the lack of a fully functional Ca^{2+}-binding site. Similarly, the originally proposed adhesive surface may have resulted from crystal packing forces, and the true nature of this region is still unknown. More recent evidence suggests that Trp2 actually inserts into a hydrophobic pocket in its own domain and that the main lateral contacts between partners are in the region of the Ca^{2+}-binding site. It remains to be seen whether all of the variant cadherin types described below can form lateral dimers, and hence we have represented cadherins as monomers in Figs 1-3 unless there is direct evidence for dimerisation (and for simplicity). An interesting question that remains to be addressed through structural studies is the role of the cleavable propeptide of classical cadherins in preventing their adhesive activity (Ozawa and Kemler, 1990). Additional residues at the N-terminus of cadherin domains seem to have a considerable effect on the structure of the molecule. The propeptide might act by preventing the docking of Trp2 into its acceptor pocket, thus preventing assumption of the adhesive conformation, but other interpretations are possible.

The role of Ca^{2+} is also somewhat disputed. Several structural and functional studies suggest a major role for Ca^{2+} in lateral interactions (see above, and Tomschy et al., 1996), others favour a role for Ca^{2+} in head-to-head trans interactions (Chitaev and Troyanovsky, 1998; Shan et al., 2000; Pertz et al., 1999). The differences between the Ca^{2+} dependencies seen may reflect genuine variations in the mechanism of different cadherins. More promiscuous lateral cadherin complexes mediated by cadherin repeats 3 and 4 can be formed in the absence of Ca^{2+} (Troyanovsky et al., 1999), but whether these are physiological is questionable because cadherins might never see such a low extracellular Ca^{2+} environment in vivo. In addition to lateral cadherin-repeat interactions, there may be contributions to dimerisation from the transmembrane regions (Huber et al., 1999) and the non-catenin-binding juxtamembrane region that binds to the armadillo family protein $p120^{ctn}$, although the effect of $p120^{ctn}$ binding seems to vary between cadherin subtypes (Ozawa and Kemler, 1998; Yap et al., 1998).

Classical cadherins interact across the intercellular gap primarily in a homophilic fashion, residues in the most N-terminal cadherin repeat being crucial (Fig. 2A) but other domains also possibly playing a role (Leckband and Sivasankar, 2000). The well known His-Ala-Val sequence and residues surrounding it contribute to adhesive binding and specificity. The alanine residue of this motif is the most highly conserved residue between cadherins, owing to its involvement in the structural fold of the cadherin domain rather than in adhesive contacts (Shapiro et al., 1995). However, more and more cadherins, such as the desmosomal cadherins (Chitaev and Troyanovsky, 1997; Marcozzi et al., 1998), are being found to form heterophilic interactions both laterally and in trans. The supramolecular organisation of cadherins also appears to be essential for strong cell-cell adhesion (Tomschy et al., 1996; Yap et al., 1997a; Yap et al., 1997b). This reflects the weak intrinsic head-to-head affinity of cadherin N-terminal domains, which thus requires cooperative interaction to generate enough adhesive strength to maintain cell adhesion. Although the linear zipper model based on the first crystal structure (Shapiro et al., 1995) provided a molecular explanation for clustering that has been useful as a framework for developing ideas, it is not now thought to be correct in detail. Alternative arrangements of cadherins, such as in cylinders (Yap et al., 1997a), are also possible.

Cadherin interactions with the cytoskeleton

The participation of β-catenin in the Wnt signalling pathway and its activation of transcription through binding to TCF/LEF proteins, as well as β-catenin stability and degradation via glycogen synthase kinase 3 (GSK-3), have been well documented and reviewed (Bienz, 1999; Brown and Moon, 1998; Gumbiner, 1998). More recently another link to cell signaling has been unraveled (Fig. 3). IQGAP1, a target of the Rho family of small GTPases that includes CDC42 and RAC1, has been shown to interact with the cytoplasmic portion of E-cadherin as well as with β-catenin. IQGAP1 and α-catenin compete for an overlapping binding site in β-catenin, and this can lead to dissociation of α-catenin from the adhesive complex. In this manner the RHO GTPases regulate cadherin-based cell adhesion and linkage of cadherins to the actin filament network, which is responsible for strong adhesion (Fukata et al., 1999; Kaibuchi et al., 1999; Kuroda et al., 1998; Vasioukhin et al., 2000). IQGAP1 also binds to calmodulin, and disruption of this interaction enhances association of IQGAP1 with the cadherin–β-catenin complex; this leads to impaired E-cadherin-mediated cell adhesion (Li et al., 1999).

Recent work (Ho et al., 1999) has shown that, in the absence of Ca^{2+}, IQGAP1 binds stably to CDC42 and inhibits its GTPase activity but that elevation of Ca^{2+} abrogates this inhibitory effect of IQGAP1 on CDC42 GTPase by binding to IQGAP1 without dissociating the CDC42-IQGAP1 complex. In addition, Ca^{2+}-calmodulin dissociates IQGAP1 from CDC42 and can also compete with IQGAP1, via its C-domain, for binding to actin filaments. Hence, IQGAP1 could couple CDC42 to actin filaments at low intracellular Ca^{2+} levels, and this coupling would be disrupted at two points after elevation of intracellular Ca^{2+}. Thus E-cadherin adhesion can be regulated by pathways that respond to extracellular signals through small GTPases and Ca^{2+} elevation, IQGAP1 acting as the adaptor. Because IQGAP1, depending on its availability at sites of adhesion, can both promote adhesion (Fig. 3A) or inhibit it (Fig. 3B), it can act as a sensitive regulator of cadherin-based adhesion. IQGAP1 availability will depend both on its expression level and on its complex interactions with regulatory proteins such as calmodulin.

VE-cadherin is a somewhat unusual member of the classical cadherin subfamily (Dejana et al., 1999). It is present at similar levels to N-cadherin in endothelial cells but, whereas N-cadherin is diffusely distributed over the cell membrane, VE-cadherin is localised to adherens junctions. This endothelial-specific cadherin is structurally highly related to other classical

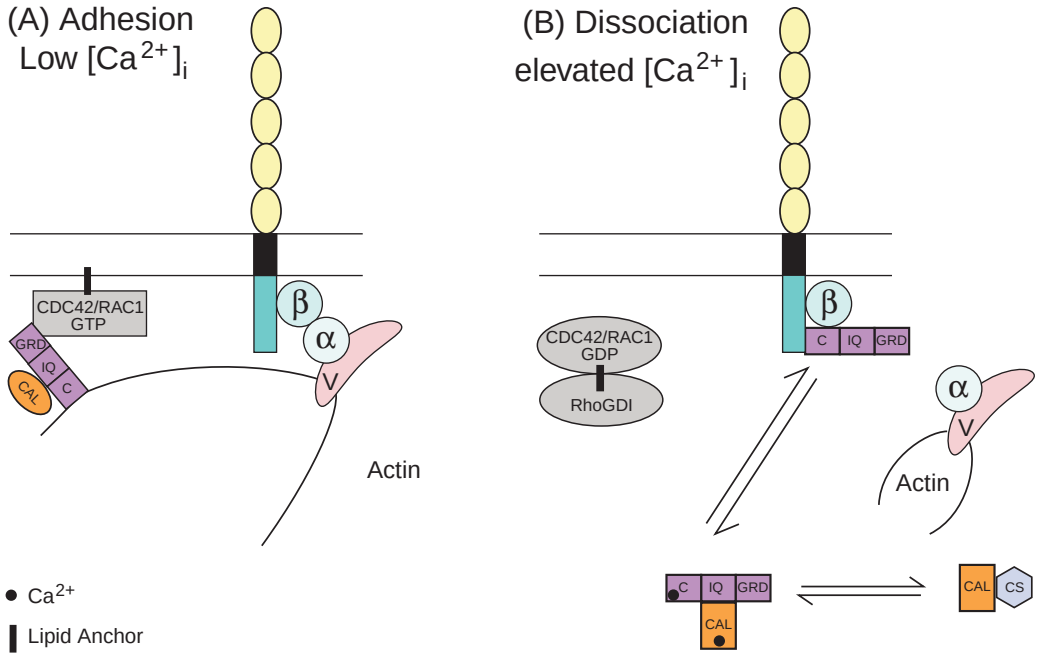

Fig. 3. Model of IQGAP1 involvement in cadherin-based cell adhesion. (A) Under conditions of low intracellular [Ca^{2+}], IQGAP1 (shown in purple) interacts with GTP-bound, active CDC42/RAC1 anchored to the cell membrane through lipid modification and located at sites of cell-cell interaction. This allows stabilisation of the cadherin-catenin-actin microfilament complex and strong intercellular adhesion. (B) On elevation of intracellular [Ca^{2+}], Ca^{2+} binds directly to IQGAP1 and to calmodulin (CAL), causing dissociation of IQGAP1 from CDC42/RAC1, which, in the GDP-bound inactive form, binds to RhoGDI. IQGAP1 can now bind to cadherin and β-catenin (β) and, by competing out α-catenin (α) binding, lead to reduced cadherin-mediated cell-cell adhesion. Calmodulin antagonists (e.g. CGS9343B; CS) release IQGAP1, which can then bind to cadherin–β-catenin and thus contribute to weakened cell adhesion. GRD, GAP-related domain; IQ, calmodulin-binding domain; C, calponin-homology domain; V, vinculin. For simplicity the cadherin is shown as a monomer.

cadherins and has five extracellular calcium-binding domains and links to the actin filament network through the catenins (Fig. 1). At very early stages of development, it is expressed by the mesodermal cells of the yolk sac mesenchyme and becomes restricted to the peripheral layer of blood islands, which subsequently gives rise to endothelial cells. VE-cadherin has multiple possible cytoplasmic binding partners; it binds to β-catenin and thus allows a link to α-catenin and the actin filament network and a connection to the Wnt signalling pathway. In addition, it interacts with $p120^{ctn}$, another member of the armadillo family, which includes β-catenin and PG (Yap et al., 1998). Interestingly, VE-cadherin can also bind to PG, which in turn binds and recruits the desmosomal plaque protein desmoplakin (DP) to the cell surface. In endothelial cells, DP co-localizes with the intermediate filament protein vimentin. This suggests a novel adhesive complex in endothelial cells, namely one that links VE-cadherin via PG and DP to the intermediate filament network (Kowalczyk et al., 1998); desmosomal cadherins (discussed below) usually form the link to the intermediate filament network. VE-cadherin seems to enable endothelial cells to form two types of membrane-bound adhesive complex (see Fig. 1) and through these to form contiguous networks either with the actin or intermediate filament networks throughout the endothelium (Dejana et al., 1999).

VE-cadherin plays an important role in vasculogenesis and vascular remodeling. Absence or intracellular truncation of VE-cadherin still allows endothelial cells to assemble to form vascular plexi (networks), which indicates that VE-cadherin-mediated endothelial homophilic interaction is not essential for this process (Gory-Faure et al., 1999). However, both have severe effects on vasculogenesis and lead to early embryonic lethality at gestation day 9.5 in mice, a consequence of vascular insufficiency. This is due to failure of endothelial cells to respond to survival signals induced by vascular endothelial growth factor type A (VEGF-A). In the absence of VE-cadherin or its cytoplasmic tail, the complex consisting of VE-cadherin, β-catenin, phosphoinositide 3-kinase (PI3-K) and VEGF receptor 2 fails to form. Consequently, VEGF-A cannot activate the serine/threonine kinase PKB/AKT or increase levels of BCL2, both part of the antiapoptotic machinery (Gory-Faure et al., 1999; Carmeliet et al., 1999; Nunez and del Peso, 1998). VE-cadherin also plays a key role in vascular permeability. The VE-cadherin–β-catenin complex is a target of permeability-increasing agents. Recent work on human umbilical vein endothelial cells has shown that the cytosolic nonreceptor protein tyrosine phosphatase SHP2 is in the VE-cadherin complex and specifically associates with β-catenin. Thrombin induces tyrosine phosphorylation of SHP2 by SRC family kinases, which leads to dissociation of SHP2 from the VE-cadherin–β-catenin complex together with phosphorylation of PG and $p120^{ctn}$ and, consequently, to reduced cell adhesion and increased cell permeability (Ukropec et al., 2000). Antibodies to VE-cadherin have been shown to prevent endothelial cell tube formation. VE-cadherin binds to fibrin domain b15-42, known to be relevant in vascular tube formation, and this unusual cadherin interaction appears to be important for fibrin-induced angiogenesis (Bach et al., 1998).

Desmosomal cadherins

The desmosomal cadherins (Figs 1 and 2B) are the transmembrane protein components of desmosomes, which are sites of cell-cell adhesion present particularly in tissues subjected to mechanical strain (e.g. epithelia, particularly

epidermis, and the myocardium). There are two subfamilies of desmosomal cadherins, the desmocollin (DSC) and desmoglein (DSG) proteins, and each possess three subtypes which are expressed in a cell-type- and differentiation-specific manner (King et al., 1997). Both subfamilies, like the classical cadherins, have five extracellular calcium-binding domains, although the fifth domain is less well conserved. In L-cells that do not normally express desmosomal proteins, the combination of a DSC and a DSG together with PG is necessary for efficient cell-cell adhesion (Marcozzi et al., 1998). In vitro antisense experiments against DSC2 (Roberts et al., 1998), expression of a dominant negative DSG (Allen et al., 1996; Serpente et al., 2000) and knocking out of the *DSG3* gene (Koch et al., 1997) all resulted in a decline in the number of desmosomes, as well as increased asymmetry, loss of ultrastructure and detachment of desmosomes. In short, a DSC and a DSG in combination mediate adhesion through their extracellular parts by engaging in homophilic/heterophilic interactions with apposed cells (Chitaev and Troyanovsky, 1997).

Desmosomal cadherins have distinct cytoplasmic regions, including a cadherin-related region, the catenin-binding C domain, through which they interact with their cytoplasmic binding partners. Each DSC has an additional shorter splice variant lacking the C domain; the function of this variant is unknown (Collins et al., 1991; Arnemann et al., 1991; Parker et al., 1991; Wheeler et al., 1991). Although the interactions with the cytoplasmic plaque have not been fully characterised, the following links emerge. PG binds to the C domain of DSGs and DSCs through its armadillo repeats (Chitaev et al., 1998; Mathur et al., 1994; Palka and Green, 1997; Smith and Fuchs, 1998; Witcher et al., 1996). PG in turn interacts with DP, which associates with intermediate filaments (Kowalczyk et al., 1997; Smith and Fuchs, 1998). Like β-catenin, PG can transduce signals to the nucleus by interacting with TCF transcription factors and is also subject to the GSK-3/APC degradation pathway (Bienz, 1999). Axin, a negative regulator of the Wnt signaling pathway, binds PG and downregulates its levels in cell culture (Kodama et al., 1999). DSG and DSC bind PG at different ratios; DSG1 at 6/7:1, DSC2a at 1:1 (Kowalczyk et al., 1996), DSG3 at 1:1 and DSG2 at <1:1 (K. J. Green, personal communication). The importance of these stoichiometric differences and the possible link between desmosomal junctions and the Wnt signaling pathway are unclear, especially since PG also localises to and functions in adherens junctions.

The armadillo family members plakophilins (PPs) can also mediate the linkage between desmosomal cadherins and the cytoskeleton (Fig. 2B), although the relative contributions of PG and PPs have yet to be fully elucidated (Hatzfeld et al., 2000; Smith and Fuchs, 1998). In contrast to PG, PPs seem to bind to DSGs, DP and keratins through their head domain (Hatzfeld et al., 2000); PP1 binds to a distinct but close site to PG in DSG1. By interacting with desmosomal proteins through its head domain, PP1 can serve as a focal point for recruiting them to the cell membrane. The molecular map of the desmosomal plaque established by immunogold localisation shows that all these links are physically possible (North et al., 1999). The armadillo repeat region of PP1 also associates with actin, induces filopodia, reduces cell contacts and stimulates the formation of motility-associated structures. Its preferred localisation in keratinocytes, however, is in desmosomes (Hatzfeld et al., 2000). The diverse association of PP1 at the crossroads of adhesion and motility could point to a role in migration, wound healing and tissue formation.

The physiological relevance of intact desmosomes and the role of DSGs within them are demonstrated by their involvement in clinical conditions. DSGs are the target antigens of autoimmune diseases in which patients show a range of blistering skin lesions – Pemphigus Vulgaris, in which DSG3 is the target antigen, being much more severe than Pemphigus Foliaceous, in which the target antigen is DSG1 (Amagai, 1999). DSG1 is also the target of proteolysis by the blister-causing exfoliative toxin A of *Staphylococcus aureus* (Amagai et al., 2000). A range of mutations in *DSG1* can cause the dominantly inherited skin disease striate palmoplantar keratoderma, in which patients show hyperkeratotic bands on palms and soles (i.e. disfiguring skin thickening). The best-characterised *DSG1* mutation is in a splice site, probably causing reduced levels of DSG1 protein and possibly also a dominant negative, truncated protein (Rickman et al., 1999). In other cases, mutations lead to extracellularly truncated proteins of various lengths, one of which encodes only 25 residues of the prosequence of DSG1 (Hunt et al., 2001). In the latter case, haploinsufficiency is the likely reason for the clinical manifestations of this disease, demonstrating the crucial importance of having correct stoichiometries of desmosomal proteins for proper function.

It is clear that the complex protein interactions emanating extracellularly and intracellularly from the membrane-bound desmosomal cadherins lead to a continuous desmosome–intermediate-filament network throughout the respective tissue that gives it stability. Whether desmosomes are involved in signal transduction is at this point uncertain but seems likely given their interaction with the armadillo family protein PG, which interacts with the Wnt pathway and regulates the expression of the anti-apoptotic protein BCL2 (Hakimelahi et al., 2000). However, these interactions might be independent of desmosome-mediated adhesion.

Protocadherins

The protocadherin family is large; members have up to seven extracellular calcium-binding domains, a single transmembrane region and divergent and distinct cytoplasmic portions. For example, the novel μ-protocadherin (Fig. 1) has four extracellular cadherin repeats, followed by an alternatively spliced mucin-like region, which can be multiply O-glycosylated, and cytoplasmic proline-rich and PDZ domains (Goldberg et al., 2000). Protocadherins generally exhibit only moderate adhesive activity (Sano et al., 1993), which is frequently not Ca^{2+} dependent. They have been found not only in vertebrates but also in a variety of lower multicellular organisms, which indicates that they may be the ancestral cadherin from which other families have evolved. Protocadherins have been strongly implicated in development; two typical protocadherins play an important role in early *Xenopus* and zebrafish development. In *Xenopus*, paraxial protocadherin (PAPC) is first expressed in Spemann's organizer, then in paraxial mesoderm and, together with axial protocadherin (APC), it partitions gastrulating mesoderm into paraxial and axial domains. Under the control of the Notch signalling pathway and the bHLH transcription factor

Thylacine1, PAPC also plays a role in maintenance of segmental gene expression and somite formation in *Xenopus* (Kim et al., 2000). These cell-type-specific homotypic interactions are also important in cell movements that drive gastrulation (Kim et al., 1998; Yamamoto et al., 1998). *PAPC* in zebrafish is a downstream target of the Spadetail transcription factor necessary for morphogenetic movements. Furthermore, the product of the homeobox gene *floating head*, which is required to block differentiation of paraxial mesoderm, represses both *PAPC* and *spadetail* expression (Yamamoto et al., 1998). In this way PAPC may form a link between transcription and morphogenesis. Furthermore, NF-protocadherin has recently been identified in the early *Xenopus* embryo in the sensorial layer of the ectoderm, and interference with its function by a dominant negative approach leads to disrupted integrity of the embryonic ectoderm (Bradley et al., 1998).

In mammals, multiple protocadherins are highly expressed in the nervous system; in fact, cadherins and particularly protocadherins are far more numerous in the brain than in any other tissue. Conservative estimates put the number of cadherins expressed in brain at >80. Recently, three clusters or families comprising a total of 52 novel protocadherins were identified on human chromosome 5q31. The protocadherins within each of these families differ in their extracellular domains but share an identical cytoplasmic domain, which gives each family a multitude of possible extracellular interactions (Wu and Maniatis, 1999). Throughout brain development, protocadherins (e.g. protocadherin-6B, protocadherin-7 and R-Cadherin) show distinct spatiotemporal expression patterns linked to other positional cues, which relate to the development of the brain into discrete segmental and functional subdivisions and provide a scaffold of adhesive clues (Arndt and Redies, 1998). Another recent example of such a protocadherin is OL-protocadherin, identified from a mouse brain cDNA library, which has a unique cytoplasmic region unrelated to any other known protocadherin and shows homophilic interaction. Its expression is restricted to a subset of functionally related brain nuclei in the main olfactory system, the limbic system and the olivocortical projections (Hirano et al., 1999). Shapiro and Colman have proposed that cadherins are the cell surface lock-and-key molecules of synaptic adhesion (Shapiro and Colman, 1999). Their structural properties fit the structure as well as the spacing of CNS synapses, and their known adhesion function and clustering in the synaptic junctions at electron-dense membrane thickenings strongly point to them as the molecules responsible for adhesion at the synapse. Interestingly, the protocadherins contain a conserved N-terminal RGD motif predicted to protrude as a loop from two secondary structure elements, which suggests that they also act as membrane-associated ligands for integrins (Shapiro and Colman, 1999).

Over the past two years a novel subfamily of cadherin-related receptors has been identified. Loss of function of the SRC family kinase FYN leads to a range of impaired behaviours, including defects in spatial learning and suckling, hyper-responsiveness to fear-inducing stimuli and enhanced susceptibility to audiogenic seizures (Yagi, 1999). A search for FYN-binding activity in mouse brain using a two-hybrid screen found eight members of a new subfamily of protocadherins called CNR cadherins or CNRs (Fig. 1). These are single-span transmembrane proteins, have a unique cytoplasmic region, carry six cadherin repeats with an RGD motif in repeat 1, and share 53-80% sequence similarity. The *FYN* and *CNR* genes exhibit overlapping mRNA expression patterns in adult mouse brain in the olfactory bulb, the hippocampus and the cerebellum. Each CNR appears to be restricted to a distinct neuronal subpopulation within those brain regions (Kohmura et al., 1998).

The large extracellular protein Reelin is produced by specific neurons, the Cajal-Retzius cells, which contain no CNRs. An antibody directed against the Reelin N-terminus disrupts the cellular arrangement of the cerebral cortex *in vitro* (Kohmura et al., 1998). Reelin can bind to the $\alpha 3\beta 1$ integrin and inhibit neuronal migration (Dulabon et al., 2000). Cadherin repeat 1 of CNR1 interacts with the N-terminal region of Reelin (Senzaki et al., 1999). In addition, the cytoplasmic adaptor protein mDAB1, primarily expressed in neurons, is a downstream target of Reelin. After tyrosine phosphorylation following binding of cortical neurones to Reelin, mDAB1 can bind to the SH2 domain of FYN; it also contains a protein-interaction domain that binds to Asn-Pro-X-Tyr motifs in the cytoplasmic tails of many receptors such as very-low-density lipoprotein (VLDL) receptor (also known as ApoER2). A monoclonal antibody recognising the Reelin-binding domain of CNR proteins blocks the signaling cascade from Reelin to mDAB1 and perturbs the arrangement of cortical neurons. Interestingly, the Reelin-binding domain of CNRs is unusually conserved, whereas repeats 2 and 3 are diverse and might therefore give individual CNRs their distinct functions (Senzaki et al., 1999).

SRC family kinase inhibitors prevent Reelin-induced tyrosine phosphorylation of mDAB1 (Howell et al., 1999). Thus, the following picture emerges: cadherin repeat 1 of CNRs can interact with Reelin and induce enzymatic activation of FYN kinase, which results in the phosphorylation of mDAB1 and consequently activation of downstream signalling pathways (Fig. 2C). One such pathway might include activation of the p35-CDK5 kinase, which associates with N-cadherin and suppresses its adhesive activity by reducing its interaction with β-catenin (Kwon et al., 2000). CNRs are thus key components of the neuronal Reelin/FYN signaling cascade and may be able to cross-regulate adhesion by classical cadherins and thus switch the adhesive specificity of neuronal cells. FYN is triply fatty acylated and known to be associated with plasma membrane lipid rafts, hot spots for cell surface signalling events in many cell types (see below and Simons and Ikonen, 1997) (Janes et al., 2000). Thus, this cascade might be activated in these specialised domains (see Fig. 2).

ARCADLIN is an exciting new member of the protocadherin family. Differential cloning techniques identified a novel gene induced by synaptic activity associated with seizures or the induction of long-term potentiation (LTP) in the rat hippocampus. The encoded protein, activity-regulated cadherin-like protein (ARCADLIN) (Yamagata et al., 1999), is a typical protocadherin; it has a single transmembrane region, six extracellular cadherin repeats and a unique cytoplasmic region, and shares 91% homology with the extracellular region of human protocadherin-8. ARCADLIN-transfected L-cells do not form cell aggregates but adhere to ARCADLIN-coated nitrocellulose dishes in a Ca^{2+}-dependent manner, which indicates weak adhesion. The protein is present in the cell

bodies and dendrites of neurons in the hippocampus and cortex in adult rat brain. In cell cultures of hippocampal neurons, ARCADLIN is found both in soma and at synapses in association with synaptophysin. Antibodies to it suppress synaptic transmission and block LTP (Yamagata et al., 1999); notably, classical cadherins also play a role in LTP (Tang et al., 1998). It will be interesting to know what intracellular interactions this neural-activity-induced cadherin has.

Seven transmembrane (7TM) cadherins

The members of a newly emerging cadherin-like protein family might be G-protein-coupled receptors (GPCRs) (Fig. 1). *Drosophila* screens have recently identified a novel type of cadherin gene called *starry night* or *Flamingo (Fmi)*. The extracellular portion of the protein consists of nine cadherin repeats but also contains EGF-like and laminin motifs, and the sequence contains predicted seven transmembrane (7TM) segments, which show similarity to GPCRs. The cytoplasmic tail lacks catenin-binding sites, and no putative cytoplasmic binding partners have been identified. This cadherin mediates homotypic adhesion (Usui et al., 1999) and is involved in the establishment of cell polarity, probably as a component of the frizzled pathway (Chae et al., 1999; Usui et al., 1999). Mouse flamingo, mFMI1, is closely related to FMI, exhibiting only eight cadherin repeats (Usui et al., 1999). Two mammalian paralogues have been found, mouse mCELSR1 (Hadjantonakis et al., 1998; Hadjantonakis et al., 1997) and human MEGF2 (Nakayama et al., 1998). Both contain eight cadherin repeats, several EGF repeats and two laminin-A G domains in their extracellular portion, and their 7TM regions again have high homology to GPCRs. Interestingly, hMEGF2 has a proline-rich sequence in its cytoplasmic portion, which could enable it to interact with SH3 domains in proteins such as non-receptor tyrosine kinases (Nakayama et al., 1998). *mCELSR1* transcription precedes gastrulation and then becomes restricted mostly to cells of ectodermal origin. In the developing nervous system it is segmentally restricted to the hindbrain as well as to dynamic dorso-ventrally restricted stripes of expression in the spinal cord (Hadjantonakis et al., 1998).

GPCRs are involved in a whole range of cellular processes, interacting with G proteins that regulate many intracellular signalling systems, including cyclic AMP, cyclic GMP, phosphoinositide turnover and ion channels. Interestingly, there is a range of human genetic disorders as well as mouse mutants in which GPCRs are altered (Coughlin, 1994). Currently, the possible interaction of 7TM cadherins with G proteins has not been tested directly. However, if this family of proteins can be confirmed as GPCRs, it will place them at the heart of cell signaling. Both GPCRs and heterotrimeric G proteins have been localised in lipid rafts or the related structures caveolae, which again raises the intriguing possibility that this group of cadherins functions in rafts.

The FAT family

Drosophila FAT is a very large protein, containing 34 tandem cadherin repeats, four EGF repeats, two laminin-A G repeats and a single transmembrane region (Fig. 1). Analysis of recessive lethal mutations in the *Drosophila fat* locus that cause larval imaginal disc overgrowth as well as differentiation and morphogenesis defects points to *fat* as a tumor suppressor gene (Buratovich and Bryant, 1997; Mahoney et al., 1991). The *Drosophila dachsous* gene is necessary for correct thorax, leg and wing development. *Dachsous* is expressed in the ectoderm of embryos and in the imaginal discs and specific regions of the brain in the larvae; the protein is highly related to FAT but contains 27 extracellular cadherin repeats. Both FAT and Dachsous proteins have cytoplasmic domains predicted to bind to β-catenin (Clark et al., 1995). Furthermore, it has recently been shown that *Dachsous* is involved in Wnt/Frizzled signalling, which is linked to β-catenin and its degradation machinery (Adler et al., 1998).

Several mammalian FAT homologues have now been identified. Human and rat FAT share 85% homology (Dunne et al., 1995; Ponassi et al., 1999). Mouse FAT has also been characterised and has high homology to other mammalian FATs, and its cytoplasmic portion interacts with β-catenin (but not plakoglobin) in a yeast two-hybrid screen (B. Cox, M. Strom and A. I. Magee; manuscript in preparation). Human MEGF1/FAT2 has only 44% homology to human FAT, has 34 extracellular cadherin repeats and a different cytoplasmic region, which does not contain a catenin-binding domain (Nakayama et al., 1998). All these family members have large extracellular domains that contain many cadherin repeats as well as EGF repeats and laminin-A G repeats. Given the size of their extracellular domains one would predict that they have roles other than cell adhesion; they appear far too large to fit into the intercellular space between closely apposed adhering cells. Furthermore, they are unlikely to have any superorganisation like that of the classical cadherins, and it is not known whether FAT family proteins are proteolytically processed. *Drosophila fat* and *dachsous* are both tumor suppressor genes, and their disruption causes imaginal disc hyperplasia. FAT family proteins are highly expressed in proliferating tissues during development and are usually less prevalent in adult tissues. Taken together, these observations could even point to a role in cell repulsion that allows cells to move and migrate for proper morphogenesis to occur. Alternatively, they could have a role as sensors of cell-cell proximity and might act as a brake on cell proliferation (Cox et al., 2000).

T-cadherin

T-cadherin was originally cloned from chick embryo brain, where it is expressed in spatiotemporally restricted patterns. It is different from all other known cadherins in that it is truncated and lacks both the transmembrane and cytoplasmic regions but instead has a GPI anchor (Fig. 1). Uncleaved precursor and the mature protein are expressed on the same cells. T-cadherin mediates calcium-dependent adhesion but is not concentrated into cell-cell contacts of transfectant cells in culture. Adhesion is ablated by treatment with phosphatidylinositol-specific phospholipase C, which removes its GPI anchor (Ranscht and Dours-Zimmermann, 1991; Vestal and Ranscht, 1992). Two recent papers suggest that T-cadherin is involved in cell signaling (Doyle et al., 1998; Philippova et al., 1998). In sucrose density gradient analysis performed on cardiac myocytes, T-cadherin-containing membrane fragments co-fractionate with markers of myocyte caveolae. However, caveolae-containing membrane fragments do not contain T-

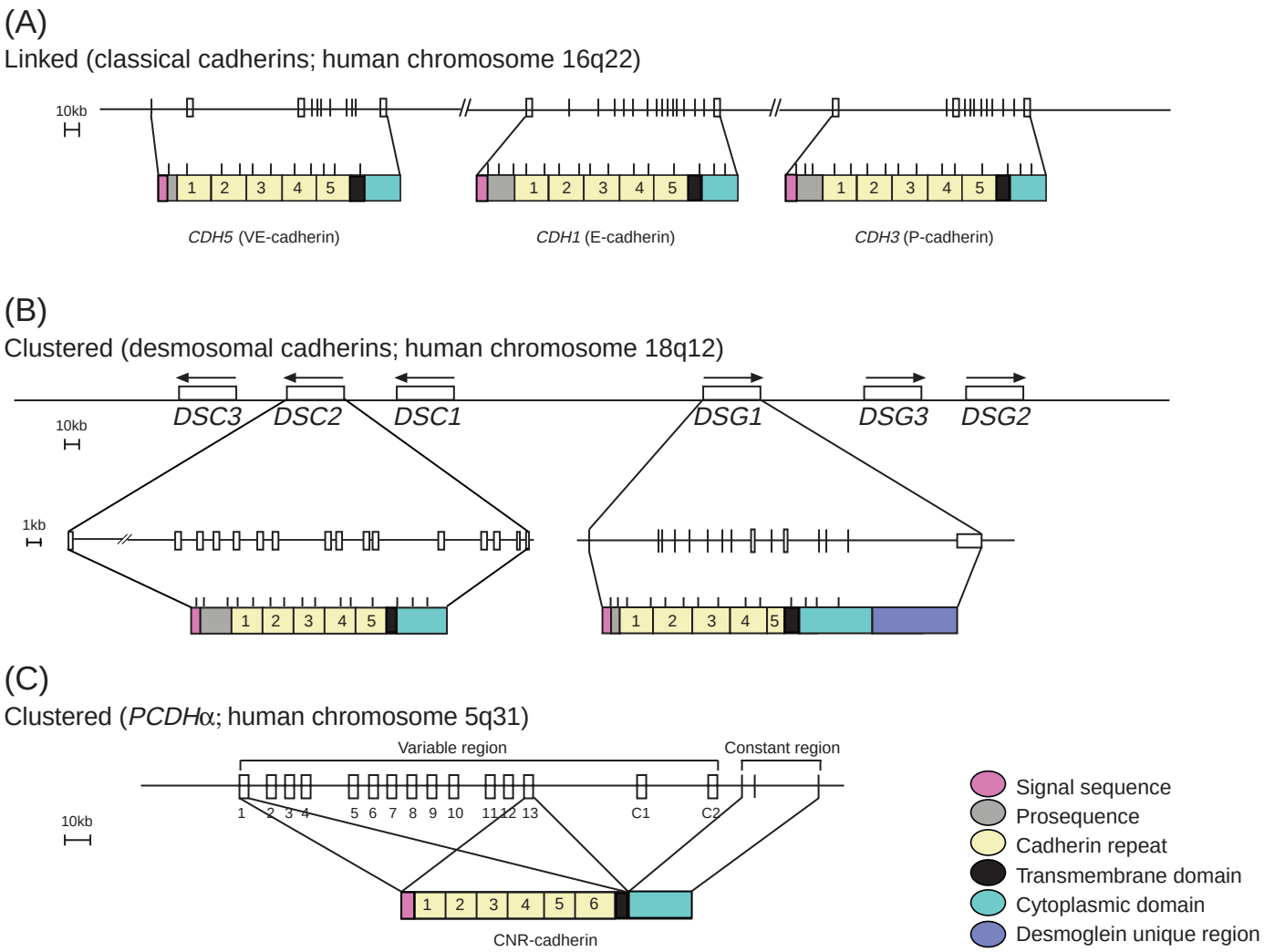

Fig. 4. Genomic organisation of cadherin genes. Although individual cadherin genes can be isolated within the genome, they frequently occur in groups that display strikingly different organisation. (A) Many of the classical cadherin genes are linked on human chromosome 16q22. They consist of 12-16 exons, and exon-intron boundaries are largely conserved. (B) Desmosomal cadherin genes, each containing 15-16 exons, are clustered on human chromosome 18q12. The correlation of their gene order and direction of transcription (indicated by arrows) points to the existence of long-range genetic elements within this gene cluster. (C) There are three protocadherin clusters (*PCDHα, PCDHβ* and *PCDHγ*) on human chromosome 5q31, which share very similar genomic organisation; the *PCDHα* (CNR cadherin) cluster is shown as an example. The extracellular (EC) region of each of the clustered protocadherins is encoded by a single uninterrupted exon differentially spliced to the cytoplasmic constant region encoded by three exons. Two examples (EC region 1 or EC region 13) are shown.

cadherin. The authors suggest that T-cadherin could be located in lipid rafts fractionating at the same density as caveolae (Doyle et al., 1998). The finding that, in vascular smooth muscle, T-cadherin can be isolated in a minor detergent-insoluble low-density membrane domain co-distributing with caveolae markers is consistent with this suggestion. Philippova et al. found that this membrane domain is enriched in other GPI-anchored proteins as well as signaling molecules such as the $G\alpha_s$ subunit and SRC family kinases (Philippova et al., 1998). Thus, the presence of T-cadherin in these domains strongly implicates it in intercellular signalling rather than adhesion per se. Perhaps its role is in 'sensing' neighbouring cells and informing its host cell of the local environment. This would be consistent with the description of T-cadherin as a negative guidance cue for neurons in the nervous system (Fredette et al., 1996).

Genomic organisation of cadherin genes

The genome organisation of the cadherins is also diverse. The genes encoding the classical cadherins E-cadherin (*CDH1*), P-cadherin (*CDH3*), VE-cadherin (*CDH5*) and KSP-cadherin (*CDH16*), as well as *CDH8*, *CDH11*, *CDH13* and *CDH15*, are located at human chromosome 16q21/22, which is syntenic with mouse chromosome 8 (see the human genome database at www.gdb.org) (Fig. 4A). Their close spatial arrangement and their conserved order might indicate that they share genetic control elements. However, the details of the intervening regions and the organisation of the linkage of these genes are currently unknown. N-cadherin shows no linkage to the other classical cadherins and is located on human chromosome 18q11.2, well separated from the desmosomal cadherin locus (Walsh et al., 1990) and from another minicluster at 18q22-q23 containing three cadherin-7-related genes (*CDH7*, *CDH19* and

CDH20) (Kools et al., 2000). This latter cluster is close to the region containing the Paget's disease locus.

Two major cadherin clusters have recently been described. The assembly of a YAC, cosmid and PAC contig of ~700 kb from human chromosome 18q12 led to the characterisation of the desmosomal cadherin gene cluster (Simrak et al., 1995; Hunt et al., 1999). The *DSC* and *DSG* genes are each about ~30 kb in size and are separated by ~25-kb intervening sequences; they form *DSC* and *DSG* subclusters separated by an interlocus region. The genes are transcribed outward from the interlocus region, and the order of the genes appears to correlate with their expression in the developing mouse embryo (see Fig. 4B); this suggests that long-range genetic elements within the cluster coordinate gene expression.

A computer-assisted approach using cadherin-like repeats to BLAST search GenBank has revealed three protocadherin (*PCDH*) clusters α, β and γ on human chromosome 5q31, including three putative *PCDH* pseudogenes (Wu and Maniatis, 1999). The orthologous region for human 5q31 has also been identified in the mouse on chromosome 18. Interestingly, this region contains the FYN-binding CNR protocadherins (Sugino et al., 2000), which correspond to *PCDHα*. In these protocadherins, diversity is generated in the 5′ (extracellular) region, whereas the 3′ (cytoplasmic) region is constant. Generation of diverse *PCDH* transcripts from these clusters might be achieved by a combination of gene rearrangement, as in the immunoglobulin and T cell receptor genes, and alternative splicing, as in immunoglobulin class switching. Each of the three *PCDH* subclusters contains at least 15 different large uninterrupted exons encoding the extracellular protocadherin region, which are spliced to an intracellular region encoded by three small exons that are separated by two large introns (see Fig. 4C). This gives these cadherins potentially diverse adhesive or cell-cell interaction properties, while maintaining association with specific intracellular partners such as FYN. The encoding of the entire extracellular portion by a single exon seems to be a common feature of protocadherins, in contrast to classical cadherins, which usually have two introns in each cadherin repeat (Wu and Maniatis, 1999; Wu and Maniatis, 2000). The high conservation of the first, fourth and fifth cadherin repeats suggests that they play important conserved roles, whereas the relatively divergent second and third repeats might define the specificity of each PCDH.

Recently it has been shown that the cytoplasmic domain of CNR-cadherins can also vary because of alternative splicing, which produces three variants (A, B and O) and the additional possibility for differential coupling to cytoplasmic partners (Sugino et al., 2000). For example, the shortest O form has no ProXXPro sequence and hence may not be able to couple to FYN through its SH3 domain. Another feature of many of the PCDH proteins is the presence of multiple cysteine residues in the cytoplasmic domains; these could be sites for S-acylation/palmitoylation and hence contribute to localisation of the proteins to lipid rafts, a known site for FYN (see above).

PCDH genes and clusters seem to have evolved by duplication (Wu and Maniatis, 2000). A second possible cluster, containing *PCDH8* and *PCDH9*, which have a very similar genomic organisation to *PCDHα*, *PCDHβ* and *PCDHγ*, is present on human chromosome 13 (Strehl et al., 1998). Thus, there may be many more related clusters, which could encode cadherins that play a role in the selective adhesion and communication that occurs in the nervous system. Interestingly, the human 5q31 region is a potential susceptibility locus for schizophrenia in German and Israeli families (Schwab et al., 1997), and *PCDH* clusters could be fertile hunting grounds for genes involved in human disease.

Conclusions

The extraordinarily diverse structures of cadherin superfamily members have adapted to perform a vast array of functions involving intercellular recognition. Frequently, this is manifested as cell adhesion, but the roles of cadherins go far beyond this, extending into cell-cell recognition, cytoskeletal organisation, signal transduction and growth control. The exciting possibility that cadherin diversity could underpin the specificity of the plethora of complex connections in the nervous system (Shapiro and Colman, 1999) will be a spur to further studies of this gene superfamily. Genomic sequence information emerging for a number of species, including *Homo sapiens*, will probably unearth yet more variations in the form and function of cadherins.

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