Cell. Mol. Life Sci. 62 (2005) 2840–2852
1420-682X/05/232840-13
DOI 10.1007/s00018-005-5320-z

Cellular and Molecular Life Sciences

Review

δ-Protocadherins: unique structures and functions

C. Redies[a,*], K. Vanhalst[b,+] and F. van Roy[b]

[a] Institute of Anatomy I, University of Jena School of Medicine, 07740 Jena (Germany), Fax: +49 3641 938512, e-mail: redies@mti.uni-jena.de
[b] Department for Molecular Biomedical Research, VIB - Ghent University Technologiepark 927, 9052 Ghent (Zwijnaarde), (Belgium), Fax: +32 (0)9 33.13.609, e-mail: F.Vanroy@dmbr.UGent.be

Received 18 July 2005; received after revision 26 August 2005; accepted 2 September 2005
Online First 2 November 2005

Abstract. δ-Protocadherins constitute a group of cadherins characterized by several conserved motifs in their cytoplasmic domains. We present a phylogenetic analysis that further divides this group into δ1-protocadherins (comprising protocadherin-1, -7, -9 and -11 or -X/Y) and δ2-protocadherins (comprising protocadherin-8, -10, -17, -18 and -19). The δ-protocadherin genes, which are located on different chromosomes in man and mouse, have a similar gene structure. They are expressed as multiple splice forms, differing mostly in their cytoplasmic domains. Some δ-protocadherins were reported to mediate weak cell-cell adhesion in vitro and cell sorting in vivo. In addition, individual δ-protocadherins might play important roles in signaling pathways, as they bind to proteins such as TAF1/Set, protein phosphatase-1α and the Frizzled 7 receptor. The spatiotemporally restricted expression of δ-protocadherins in different tissues and species and the results of their functional analysis, mainly in *Xenopus*, suggest that they play multiple, tightly regulated roles in vertebrate development.

Key words. Phylogenetic analysis; gene structure; alternative splicing; cell adhesion; intracellular signalling; expression pattern; protocadherins.

Introduction

Cadherins are cell adhesion receptors that contain multiple 'cadherin repeats' of about 100 amino acids in their extracellular domains. More than 100 different cadherin genes have been identified in the vertebrate genome and classified into several subfamilies, including classic cadherins (type I and type II), protocadherins and desmosomal cadherins [1, 2]. The largest subfamily, the protocadherins, has grown considerably during the last decade [3]. Several subgroups of protocadherins have been identified, such as the α-, β- and γ-protocadherins, which are encoded by three gene clusters on chromosome 5q31 in the human genome. Other subgroups are the flamingo (CELSR) cadherins, and the large (Fat- and Dachsous-related) protocadherins.

In this work, we present a phylogenetic analysis of all mouse and human protocadherin subgroups. Results support the identification of a novel subgroup of protocadherins, which we named δ-protocadherins [4]. This subgroup comprises at least nine protocadherins (Pcdhs), all of which contain highly conserved motifs

* Corresponding author.
+ Present address: European Patent Office, Patentlaan 2, 2280HV Rijswijk (The Netherlands), e-mail: kvanhalst@epo.org

in their cytoplasmic domains [4, 5]. Based on several features discussed in detail below, δ-protocadherins can be further subdivided into two groups, the δ1-protocadherins (protocadherin-1, -7, -9 and -11) and the δ2-protocadherins (protocadherin-8, -10, -17, -18 and -19) [4]. We will review the gene structure of these protocadherins, their cytoplasmic binding partners, and their roles and expression patterns in developing and adult vertebrates.

Phylogenetic analysis

The classification of δ-protocadherins proposed above is based on overall structural differences between the diverse protocadherin genes [4]. To test the reliability of this classification, we performed a phylogenetic analysis of the protein sequences of all protocadherins known in mouse and man, using the methods described previously [6]. For the large α-, β- and γ-protocadherin subgroups, we selected only four members from each subgroup in order to reduce complexity. Due to the diverse lengths of the protocadherin proteins, analysis was carried out with shorter segments of protein sequences that are similar in the diverse protocadherins. Either the cytoplasmic domains or the first two extracellular cadherin repeats were used for the analysis. Multiple alignments and phylogenetic analysis were performed using the ClustalX program [7], and the bootstrap neighbor joining trees were visualized using the Treeview program [8].

The resulting phylogenetic tree for the combined human and mouse sequences is shown in figure 1. Analyses using either the human or the murine sequences gave identical results. We therefore focus here only on the combined analysis. Also, analysis using either the cytoplasmic domains or the first and second extracellular domains yielded similar results. The results described here are based on the cytoplasmic domains.

Our analysis confirmed the existence of the following protocadherin subgroups (fig. 2) proposed previously [1–3, 9]: (i) CELSR protocadherins that contain seven-pass transmembrane domains; (ii) Fat-like protocadherins proteins with far more than seven extracellular domains; (iii) the protocadherin-α, -β and -γ subgroups, each of which has a specific genomic organization, and which are clustered in a small genomic region. Other protocadherins (e. g. Mu-protocadherin and protocadherins-12, -15, -20 and -21, and Dachsous 1 and 2) are rather solitary members in the tree, indicating that they differ in origin despite the similarity of their domain structures to those of other cadherins. Remarkably, protocadherin-12 forms a small subgroup with Mu-protocadherin, although the two genes are structurally

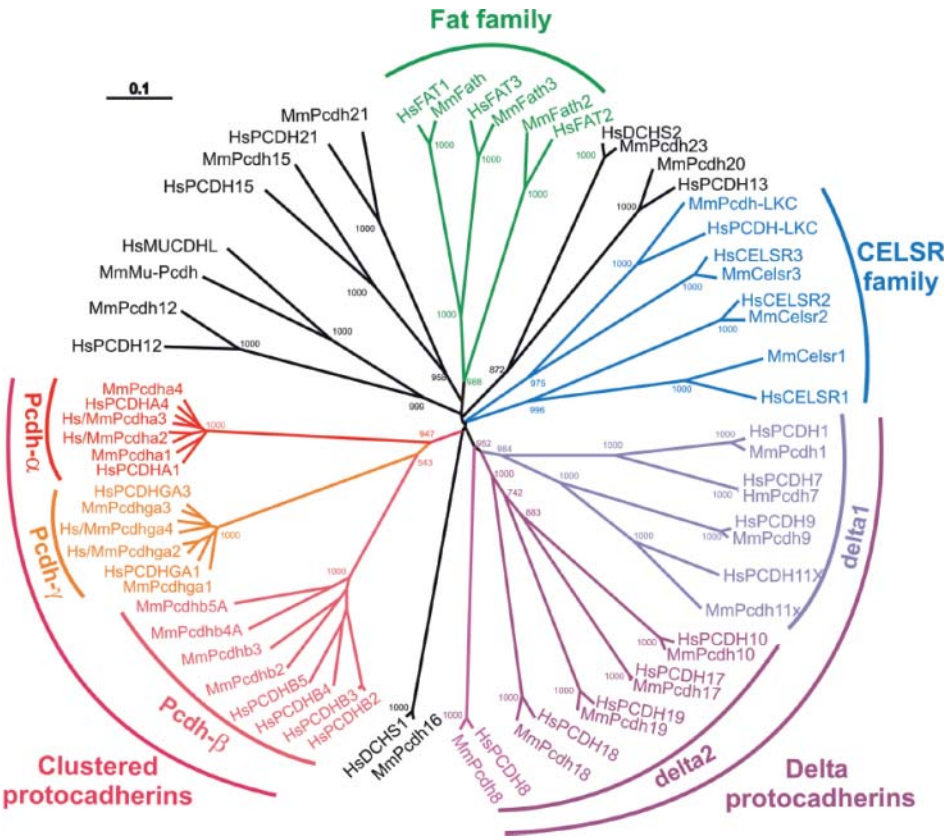

Figure 1. Phylogenetic analysis of human (Hs) and mouse (Mm) protocadherins on the basis of their aligned cytoplasmic domains. Four families can be discerned: the δ-protocadherins, divided into δ1- and δ2-protocadherins; the clustered protocadherins, divided into the α-, β- and γ-protocadherins; the CELSR or Flamingo-like family; and the Fat family. Other protocadherins are more difficult to place, although some of them show significant similarity (e. g. Pcdh15 and Pcdh21). Abbreviations: CELSR, cadherin EGF LAG seven-pass G-type receptor; DCHS, dachsous-related protocadherin; MUCDHL, mucin- and cadherin-like protocadherin. The most recent official human and mouse gene nomenclatures were adopted except for a more informative one in the protocadherin-β subfamily [6]. Bootstrap values indicate the likelihood of the branching (values higher than 700 point to a probability of more than 95%). The length of the bar (top left) corresponds to 1 amino acid substitution per 10 residues.

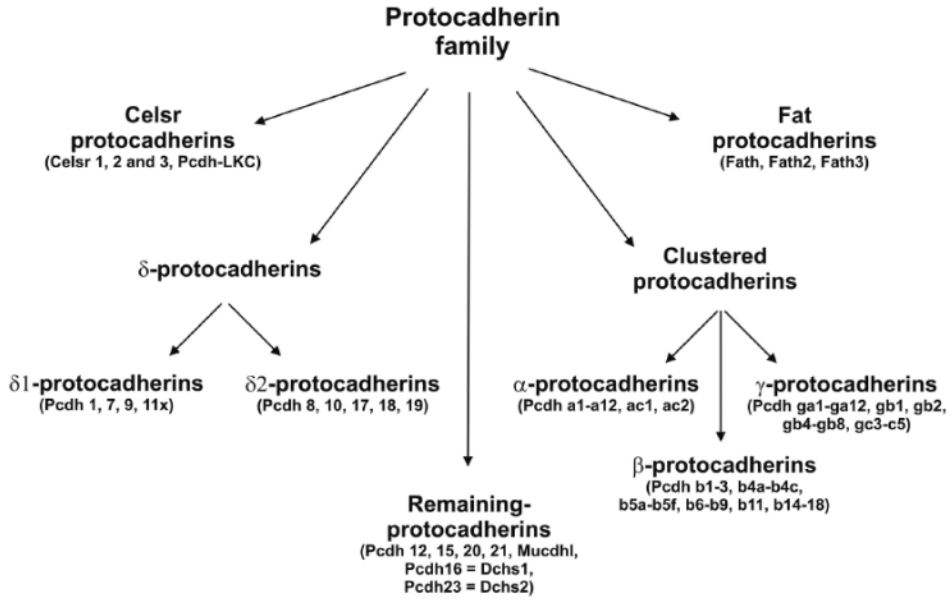

Figure 2. Simplified scheme of the different protocadherin subgroups with representative mouse members listed in parentheses.

very different. Possibly, similarities in their cytoplasmic domains may explain this rather unexpected result. It is interesting to note that the Dachsous-like protein protocadherin-16 (DCHS1) [10], and the structurally related Dachsous-like protein protocadherin-23 (DCHS2) [11], each of which contains 27 extracellular calcium-binding cadherin (EC)-repeats, have very distinct positions in the tree. This is less surprising given their totally different gene structures in the human genome (http://www.ensembl.org/Homo_sapiens/), pointing to a convergent evolution of the two genes. Moreover, neither of the Dachsous-related proteins is included in the Fat family, which was previously believed to comprise all large protocadherins. Another large protocadherin, Pcdh15, contains 11 EC repeats and also appears to be a solitary member. Recently another member of the human FAT-protocadherin family was reported, namely FAT4 [11]. Since the messenger RNA (mRNA) and protein sequences of the mouse FAT4 ortholog were still preliminary at the time of our analysis, we did not include it. The human FAT4 protein sequence shows the highest homology with human FAT1.

Phylogenetic analysis identified a novel subfamily of protocadherins that comprises nine members (Pcdh1, 7, 8, 9, 10, 11, 17, 18 and 19). All of these protocadherins contain the previously identified amino acid motifs CM1 and CM2 [5] in their cytoplasmic tails, indicating that they indeed belong to the same subfamily (δ-protocadherins) [4]. The present review is focused on this novel subfamily.

Two subgroups of δ-protocadherins

The subfamily of δ-protocadherins (table 1) can be divided into two groups that are clearly delineated in all the phylogenetic analyses performed in the present work. The first subgroup (δ1-protocadherins) comprises protocadherin-1, -7, -9 and -11(X/Y) (Pcdh1, Pcdh7, Pcdh9 and Pcdh11[X/Y]). These are the only known protocadherins with seven extracellular cadherin domains [1, 2, 4, 12, 13]. In addition to the CM1 and CM2 motifs, the cytoplasmic domains of δ1-protocadherins contain a small but highly conserved motif (RRVTF), which we designated CM3 [4]. This motif is necessary for the binding of each of the four δ1-protocadherins to protein phosphatase-1α (PP1α; see below).

The second subgroup (δ2-protocadherins) comprises protocadherin-8, -10, -17, -18 and -19 (Pcdh8, Pcdh10, Pcdh17, Pcdh18 and Pcdh19). These proteins contain a CM2 motif and a CM1 motif, the latter of which is more highly conserved in δ2-protocadherins than in δ1-protocadherins [4, 5]. The CM3 motif is absent. Unlike δ1-protocadherins, all δ2-protocadherins have six extracellular domains.

Structure of the δ-protocadherin genes and alternative splicing

The δ-protocadherin genes share several features that discriminate them both from genes encoding classic cadherins and from genes encoding clustered protocadherins (-α, -β, -γ), as illustrated in figure 3 [4, 6, 9, 14]. In contrast to the EC of classic cadherins, whose transmembrane regions and cytoplasmic domains are encoded by numerous smaller exons (exemplified by the E-cadherin or Cdh1 gene in fig. 3A), protocadherin genes as a rule comprise quite a large exon, which encodes most if not all of the extracellular domain, a transmembrane

Table 1. δ-Protocadherin genes.

Name	Human gene symbol	Synonyms or orthologous genes	Species[a]	Interaction partner	Reference	Human/mouse gene locus[b]
				δ1-Protocadherins		
Protocadherin-1	PCDH1	protocadherin-42	Hs, Mm	PP1α	[4, 17]	5q31.3/18qB3
		axial pcdh (AXPC)	Xl		[21, 22]	
Protocadherin-7	PCDH7	BH-protocadherin	Hs, Mm	PP1α	[4, 49, 53]	4p15.1/5qC1
		NF-protocadherin	Xl	TAF1/Set	[23, 29]	
Protocadherin-9	PCDH9	protocadherin-9	Hs, Mm	PP1α	[4, 55]	13q21.32/14qE2.1
Protocadherin-11	PCDH11X	protocadherin-X	Hs, Mm	PP1α	[4, 12]	Xq21.3/XqA1.1
	PCDH11Y	protocadherin-Y	Hs		[13]	Yp11.2/-
	PCDH11Y	protocadherin-PC	Hs	β-catenin	[78]	
				δ2-Protocadherins		
Protocadherin-8	PCDH8	protocadherin-8	Hs	?	[55]	13q14.3/14qD3
		arcadlin	Rn		[19]	
		paraxial pcdh (PAPC)	Xl	Xfz7	[22, 32]	
Protocadherin-10	PCDH10	KIAA1400	Hs	?	[72]	4q28.3/3qB
		OL-protocadherin	Mm, Gg		[75, 76]	
Protocadherin-17	PCDH17	protocadherin-68	Hs, Rn	?	[1]	13q14.3/14qD3
Protocadherin-18	PCDH18	KIAA1562	Hs, Mm	mDab1	[40, 79]	4q28.3/3qC
		protocadherin-68-like	Hs		[5]	
Protocadherin-19	PCDH19	KIAA1313	Hs	?	[72]	Xq13.3/XqE3
			Hs		[5]	

[a] Gg, *Gallus gallus*; Hs, *homo sapiens*; Mm, *mus musculus*; Rn, *Rattus norvegicus*; Xl, *Xenopus laevis.*
[b] Chromosomal location on the basis of the literature and the human and mouse genome projects (URL: http://genome.ucsc.edu/index.html)

region and part of the cytoplasmic domain (fig. 3B–E). Other points of divergence are the lack of a prodomain in protocadherins and the frequent occurrence of alternative splice variants. Notable exceptions to the latter rule are the β-protocadherin genes, which form a cluster of up to 22 one-exon genes (fig. 3C). For the clustered α- and γ-protocadherin genes, one can discern three types of splice variants: O, A and B (fig. 3B). Isoform O originates from a readthrough of the larger exon into the 3' intron. The same phenomenon is regularly observed in the δ-protocadherin genes (fig. 3D, E), and leads to a truncated cytoplasmic domain. Isoforms A and B of the α- and γ-protocadherins are generated by splicing one of numerous variable exons #1 to a set of constant exons (#c1 to #c3). δ-Protocadherin genes show similar patterns of alternative splicing, although in this family all genes have unique exons, not shared by related family members. Moreover, the encoded cytoplasmic domains of δ-protocadherins show mutual sequence similarity due to the presence of conserved motifs (CM1–CM3) as discussed above, and differ significantly from the cytoplasmic domains of clustered protocadherins. If CM3 is present (δ1-protocadherins), it is invariably encoded by the same exon as CM1, whereas CM2 is encoded each time by the 3'-most exon. Alternative intraexonic splicing does occur in several δ-protocadherin genes (exemplified by isoform 4 of Pcdh9 in fig. 3D) [4]. Two of the nine δ-protocadherin genes, i.e. Pcdh11 and Pcdh19, both located on the X-chromosomes of man and mouse (table 1), are expressed as a surprisingly large collection of splice variants, due to the alternative use of several small exons encoding either the 5' untranslated region or parts of the highly variable cytoplasmic domain (unpublished results and [4, 15]). Figure 3E depicts only 5 variants out of the 10 reported so far for mouse Pcdh11. Differential expression patterns of alternative splice variants of δ-protocadherins have been reported for Pcdh8 [16] and Pcdh11 [15]. However, hardly anything is known about the functional implications except for the fact that δ1-protocadherin isoforms lacking CM3 are unable to associate with PP1α [4] (see below).

Protocadherins mediate cell-cell adhesion

δ-Protocadherins resemble classic cadherins in their ability to mediate cell adhesion, although binding appears to be generally weaker. In particular, in vitro adhesive activity has been demonstrated for Pcdh1 [17], Pcdh7 [18], Pcdh8 [19] and Pcdh10 [20]. Moreover, by preferring homophilic over heterophilic interactions, some δ-protocadherins are able to mediate cell sorting in cell culture (e.g. Pcdh1 [17] and Pcdh10 [20]) and can do this in vivo as well. For example, axial protocadherin (AXPC, a Pcdh1 ortholog) and paraxial protocadherin (PAPC, a Pcdh8 ortholog) mediate the sorting of paraxial and prenotochordal mesenchyme during *Xenopus* gastrulation [21, 22] (see below). NF-protocadherin, a Pcdh7 or-

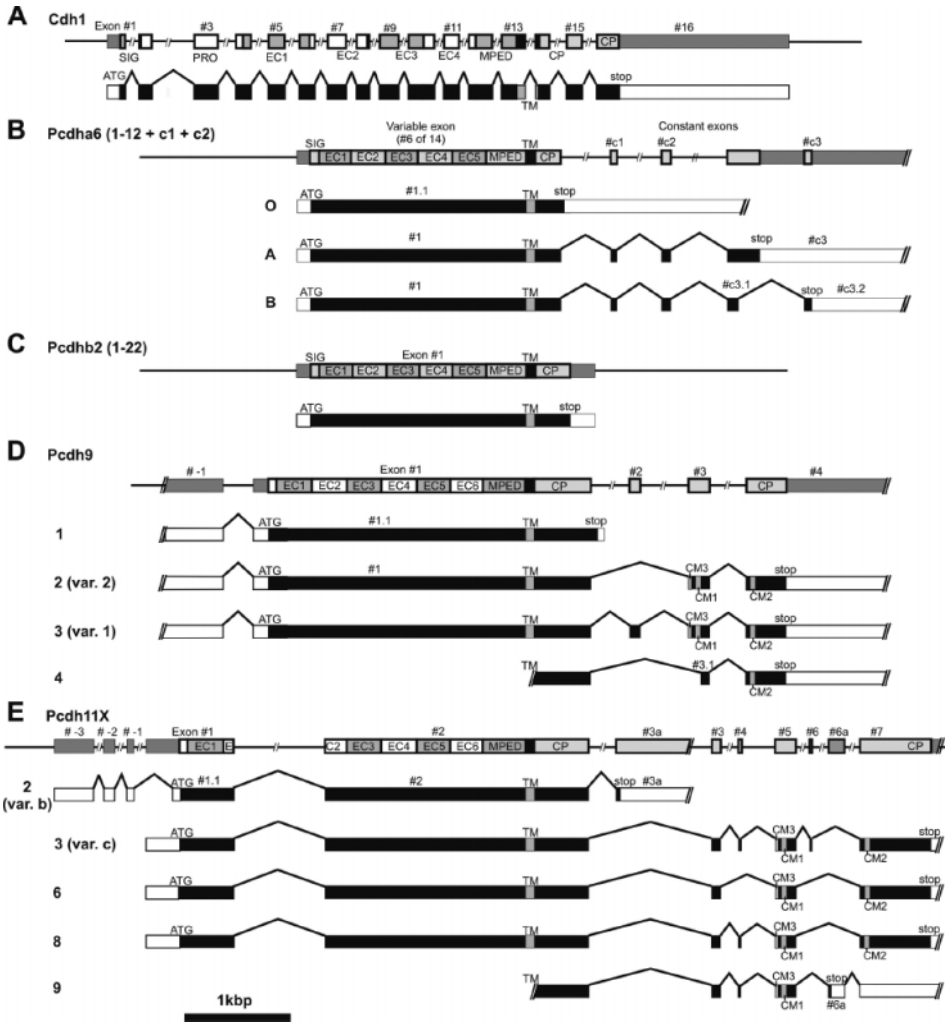

Figure 3. Gene structure and alternative transcripts of representative members of mouse cadherins and protocadherins. In each panel the gene is depicted at the top followed by typical transcripts. Numbered boxes represent exons; the intervening line represents intronic sequence (the latter is not to scale). Sequences encoding protein domains are shaded differently and designated: SIG, signal peptide; PRO, prodomain; EC, extracellular cadherin repeat; TM, transmembrane domain; MPED, membrane-proximal extracellular domain; CP, cytoplasmic domain; CM3, CM1 and CM2, conserved motifs. In the transcripts black boxes represent coding sequences, white boxes represent 5' or 3' untranslated regions. Transcript variant annotations refer to the literature [4, 14]. Exon #1 is defined as the exon that contains the standard start codon (indicated by 'ATG'); alternatively used exons are indicated by the extension 'a'; exons that are elongated by readthrough in the neighboring intron or that become shortened by an intraexonic splice acceptor are indicated by the extension '.1' or '.2'. Exons that are shared by a cluster of genes are designated 'c' (constant). Transcripts indicated with an asterisk are the standard or full-length transcripts. (A) Cdh1 gene encoding E-cadherin as paradigm for classic cadherins. Note the high number of smaller exons, none of which encodes a protein domain. (B) Pcdhα6 gene encoding protocadherin-α6 as paradigm for the clustered protocadherin-α and -γ genes. In mouse, the 14 Pcdh-α genes are numbered 1–12 plus c1 and c2, each with a long and unique exon #1, which encodes a complete but short protein isoform in the case of transcript variant O. Transcripts A and B encode larger cytoplasmic domains and are generated each time by splicing of variable exon #1 to the same set of shared constant exons. Transcript B shows alternative splicing within exon #c3. (C) Pcdhb2 gene encoding protocadherin-β2 as paradigm for the clustered protocadherin-β genes. These genes differ from the other clustered Pcdh genes by the lack of shared, constant exons. (D) Pcdh9 gene encoding protocadherin-9 as paradigm for most δ-protocadherin genes. Transcripts: 1, short transcript similar to transcripts O of the clustered protocadherin genes; 2, standard transcript; 3, transcript with additional exon #2; 4, transcript with variant exon #3.1, lacking CM3 and CM1. (E) Protocadherin-11 gene and a selection of the numerous encoded transcripts: 2 or b, use of alternative exon #3a; 3 or c, full-length standard transcript; 6, skipping of both exon #4 and exon #6; 8, skipping of only exon #6; 9, use of alternative exon #6a, resulting in premature stop codon and loss of CM2. The black bar represents a distance of 1 kbp.

tholog, can mediate the sorting of cells in the embryonic epidermis of *Xenopus* in a homotypic manner, but not in the absence of the intracellular domain [23].

Protocadherins and intracellular signaling

Several recent reports have made it clear that protocadherins are probably more involved in intracellular signaling than in strong cell-cell adhesion (fig. 4). The homophilic interaction capacities of the protocadherins tested so far indicate that specific recognition may be the first step in signaling. This initial recognition might then trigger downstream intracellular signaling, a hypothesis based on the identities of the interaction partners that have been identified for several protocadherins. Most of these cytoplasmic binding partners are members of well-known signaling pathways. For example, the longest isoform of human Pcdh7 has been reported to interact with protein phosphatase-1α (PP1α; fig. 4A). PP1α is

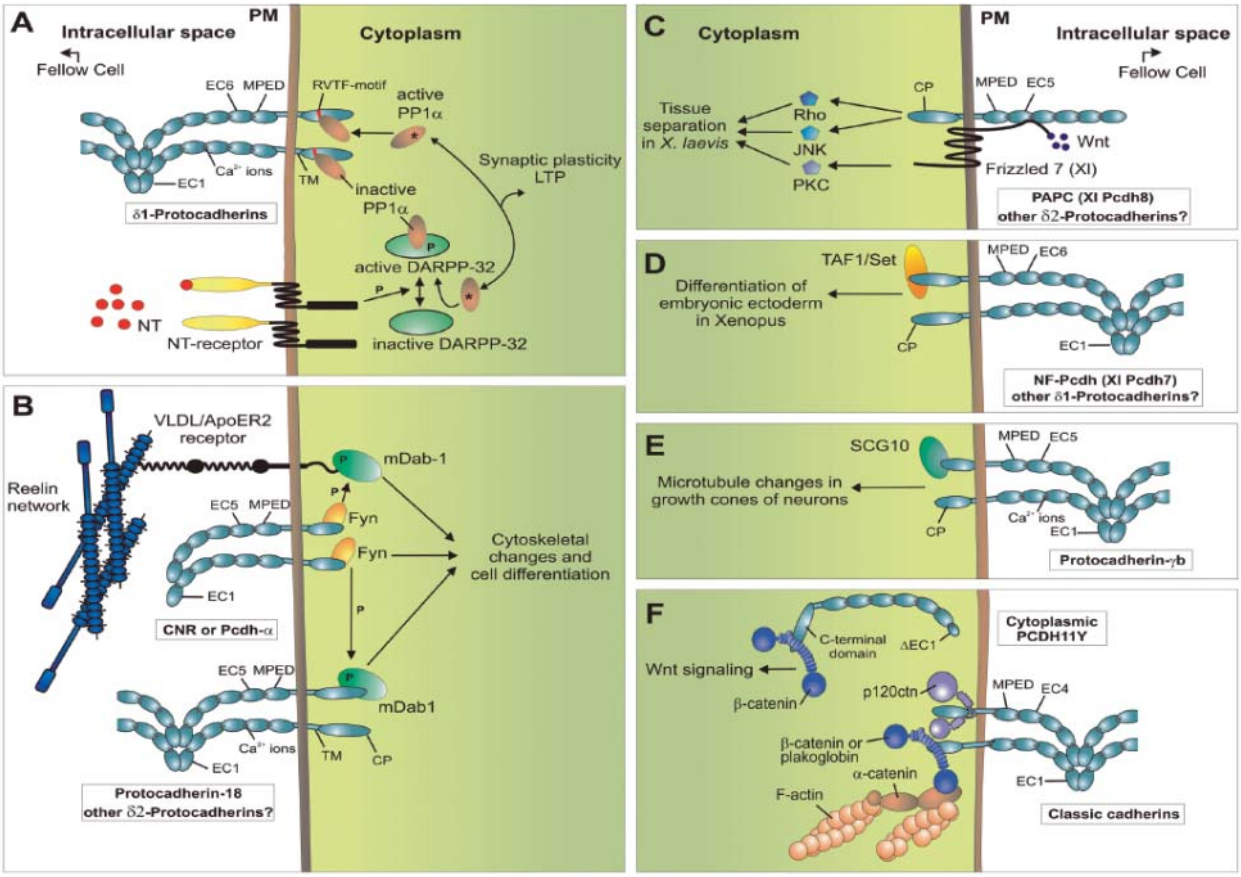

Figure 4. Schematic overview of intracellular signaling pathways known to involve protocadherin. From top to bottom: (A) δ1-protocadherins interact with the PP1α protein, possibly inactivating it. They might work in synergy with neurotransmitters to regulate synaptic plasticity. (B) Members of the CNR or protocadherin-α family interact intracellularly with the Src-related protein kinase Fyn, which is involved in phosphorylation of mDab-1. Both Fyn and mDab-1 have been linked to intracellular pathways leading to cytoskeletal changes, neuronal differentiation and correct architecture of cortical layers in the brain. Another protein that interacts with mDab-1 is protocadherin-18, a member of the δ2-protocadherins. It is still unclear whether other δ2-members can interact with mDab-1. (C) Paraxial protocadherin (PAPC), the *Xenopus laevis* ortholog of protocadherin-8, another δ2-protocadherin, interacts with the Frizzled-7 receptor. These two transmembrane proteins act synergistically in the process of tissue separation during convergence-extension of *Xenopus* embryos, through activation of Rho-A, JNK (PAPC) and PKC (Frizzled-7). (D) Another *Xenopus* protocadherin, NF-pcdh (ortholog of Pcdh7, a δ1-protocadherin) interacts with the histone-regulating protein Taf-1/Set, possibly leading to differentiation of *Xenopus* embryonic ectoderm. (E) Several members of the protocadherin-γ subfamily interact with the microtubule-destabilizing protein SCG10, possibly resulting in cytoskeletal rearrangements in growth cones of neurons. (F) The human PCDH11Y gene product differs from the highly related PCDH11X proteins by expressing an alternative aminoterminus lacking a signal peptide. Therefore, this protein is retained in the cytoplasm where it might interact with β-catenin and contribute to Wnt signaling and tumorigenesis. For comparison, the classic model of the cadherin-catenin complex is drawn at the bottom. Abbreviations C, carboxy-terminus; CP, cytoplasmic domain; EC, extracellular cadherin repeat (ΔEC1 is an EC1 domain with truncated amino-terminus); LTP, long-term potentiation; MPED, membrane proximal extracellular domain; N, amino-terminus; NT, neurotransmitter; P, phosphorylation reaction or site; PM, plasma membrane; TM, transmembrane domain; Xl, *Xenopus laevis*.

known to bind the consensus motif (R/K)(V/I)XF [24], which is also found in the long form of the Pcdh7 cytoplasmic domain. It was demonstrated that this domain is indeed needed for interaction with PP1α in both mouse and man [4, 25]. Interestingly, we identified this RVTF motif in the cytoplasmic regions of three more members of the protocadherin family, i. e. Pcdh1, 9 and 11 (all δ1-protocadherins), and showed that PP1α is an interaction partner of all four members of the δ1-protocadherin subfamily [4], but not of δ2-protocadherins [our unpublished data]. Earlier reports indicated that the PP1α protein is specifically enriched in medium-sized spiny neurons of the striatum [26] and concentrated in the dendritic spines of these neurons. Several neurotransmitter pathways [dopaminergic, glutamatergic and GABA (Gamma-aminobutyric acid)ergic] exercise part of their functions in these medium-sized spiny neurons by regulating the phosphorylation state of DARPP-32 (dopamine and cyclic-AMP-regulated phosphoprotein of 32 kDa). Interestingly, DARPP-32 is a very potent inhibitor of PP1α, but only when DARP32 is in the active, phosphorylated state (fig. 4A). PP1α is thought to act as a negative regu-

lator downstream of the neurotransmitter receptors [27], which has implications for the regulation of synaptic plasticity and both long-term potentiation (LTP) and long-term depression (LTD) [28]. As Pcdh7 inhibits the activity of PP1α [25], it could act synergistically with the neurotransmitter receptor-DARPP-32 signaling pathway. The cell adhesive properties of the Pcdh7 molecules coupled to its inhibition of PP1α activity, indicate that Pcdh7 could play an important role in the regulation of synaptic plasticity.

NF-protocadherin, a *Xenopus laevis* ortholog of Pcdh7, was recently reported to interact with TAF1/Set (TATA-binding protein (TBP)-associated factor-1; fig. 4D), previously identified as a histone-binding protein promoting transcriptional access to chromatin. Both NF-protocadherin/Pcdh7 and TAF1/Set are involved in ectoderm formation in the early *Xenopus laevis* embryo [29]. TAF1/Set has been shown previously to modulate Cdk5 activity, involved in neuronal differentiation, neurocytoskeleton dynamics, and neuronal degeneration and cell death [30]. TAF1/Set further inhibits demethylation of specific promoters, resulting in gene silencing. TAF1/Set is often upregulated in tumor cells, indicating that it is an oncoprotein [31]. It would be interesting to investigate whether Pcdh7 is also involved in some of these processes.

Recently, the *Xenopus laevis* paraxial protocadherin (PAPC, a Pcdh8 ortholog) was shown to interact with the Frizzled 7 receptor (fig. 4C). Intracellularly, the PAPC/Pcdh8 and Frizzled receptors synergistically activate the small GTPase RhoA, JNK (Jun N-terminal kinase) and PKC (protein kinase C), leading to tissue separation during the convergence-extension phenomenon in *Xenopus* embryos [32, 33]. This convergence-extension process is mediated by the planar cell polarity (PCP) pathway, which has been studied extensively in *Xenopus*, zebrafish and *Drosophila*. Another interesting protocadherin, Celsr1 or Flamingo, has also been recently implicated in this pathway, working together with PCP mediators Wnt11 and Strabismus [34]. Celsr3 (murine flamingo family member) mutant mice have selective anomalies of several specific major axon fascicles, implicating this protocadherin functions in axon development in mammals [35]. In analogy to the *Xenopus* PAPC/Pcdh8 story, the neural phenotype in Celsr3 mutant mice is similar to that caused by inactivation of Fzd3, a member of the Frizzled family. Celsr3 and Fzd3 are also coexpressed during brain development and may act in synergy. This indicates that protocadherins of different subfamilies might be involved in similar or identical pathways.

Another family of protocadherins, the CNR or protocadherin-α family, interacts intracellularly with the tyrosine kinase Fyn (fig. 4B) [36], a key molecule in regulation of synaptic plasticity and LTP. The CNRs were originally believed to interact extracellularly with the ECM (extracellular matrix) molecule Reelin, but more recent studies yielded contradictory results [37]. Reelin is expressed in Cajal-Retzius cells and is involved in the positioning of cortical neurons. It is believed that it somehow signals through the CNRs and the VLDL/ApoER2 receptors, triggering intracellular signaling through Fyn, and further downstream through mDab-1 (mouse Disabled-1), an intracellular adaptor protein that regulates axon outgrowth in the developing nervous system. mDab-1 is a downstream component of the Reelin signaling pathway, which controls neural positioning in the vertebrate brain [38]. The phenotypes of mice defective in Reelin, mDab-1 or Fyn are very similar and are characterized by inverted cortical layers. A 'positive selection model' has been proposed: Reelin-expressing cells select functional neurons through their expression of specific CNRs [39]. Alike this CNR-Fyn complex, Pcdh18 has been shown to interact directly with the intracellular protein mDab1 [40].

Additionally, protocadherin-γb1 and other members of the protocadherin-γb subfamily of clustered protocadherins interact with the microtubule-destabilizing protein SCG10 (fig. 4E) [41]. Protocadherin-γb1 and SCG10 are both expressed in the growth cones of a subset of neurons in the spinal cord. Although no direct effect of protocadherin-γb1 on SCG10 protein activation or on microtubule regulation has been shown to date, this molecular interaction raises interesting questions on the role of protocadherins in neuronal growth cone organization.

Another report showed that γ-protocadherins are cleaved both extracellularly (by a matrix metalloproteinase) and intracellularly (by presenilin). The final cleavage product, comprising the C-terminal part of the cytoplasmic domain, translocates to the nucleus [42, 43]. Similarly, the cytoplasmic protein cleavage fragment of human Fat-1 contains a nuclear localization signal (NLS) and is detectable in the nucleus [44]. These reports indicate that in addition to intracellular signaling through interacting proteins, the cytoplasmic tails of several protocadherins by themselves might have a signaling function.

Expression patterns and functional roles

δ1-Protocadherins

Protocadherin-1 (Pcdh1)

Pcdh1 (previously called protocadherin-42) was one of the first protocadherins identified by reverses transcription polymerase chain reaction (RT-PCR) with degenerate cadherin primers by Shintaro Suzuki's group [17]. In transfected cells, it localizes to cell-cell junctions and mediates weak homophilic cell-cell adhesion [17, 45]. In the rat, Pcdh1 is strongly expressed in the brain [17]. Expression was found also in endothelial cells of the adult lung in the mouse [46].

An ortholog of Pcdh1 has been called axial protocadherin (AXPC) in the frog *Xenopus laevis* and zebrafish [21, 22]. The gene was identified together with paraxial protocadherin (PAPC/Pcdh8, see below) in a screen for genes that separate paraxial mesoderm and axial prenotochordal mesoderm, respectively, at the neurula stage in *Xenopus* [22]. At the tailbud stage, AXPC/Pcdh1 is also expressed in the pronephros, somites, heart, otic vesicle and distinct parts of the brain [21]. By microinjection of morpholino oligonucleotides and different truncated mRNA species, AXPC/Pcdh1 was shown to be necessary and sufficient for prenotochordal cell sorting in the gastruling embryo. Interestingly, overexpression of its extracellular domain suffices to induce cell sorting [21]. One candidate gene to regulate the expression of AXPC/Pcdh1 is the *Xenopus* organizer gene *Xlim-1*.

Pcdh1 is upregulated in a cell variant, derived from the human endothelial cell line ECV-304, and selected for resistance to p53-meditated apoptosis [47]. This upregulation coincided with the acquisition of distinct cell behavior, such as contact inhibition and formation of cyst-like structures in culture. Upregulation of Pcdh1 was also observed after treatment of cells from a skin culture model with the known skin irritant sodium lauryl sulfate [48].

Protocadherin-7 (Pcdh7)

Three different full-length complementary DNA (cDNA) isoforms of Pcdh7 (Pcdh7a, -7b and 7c) were isolated in a screen for putative transmembrane proteins from a human gastric adenocarcinoma cell line [49]. The two shorter transcripts, Pcdh7a and -7b, mediate cell-cell adhesion in mouse fibroblast L cells, while the longer transcript, Pcdh7c, which shows an intraexonic deletion and therefore lacks a Ca^{2+}-binding pocket in the extracellular domain, does not mediate cell adhesion [18]. Similar transcripts were found in mouse, but here the longer isoform did not have the deletion in the extracellular domain [4]. The longer transcripts encode an extended cytoplasmic tail, which interacts with PP1α (see above) and inhibits PP1α activity towards glycogen phosphatase [25].

Several cancer cell lines express a truncated form of Pcdh7 (4.5 kb) that is not found in normal tissue. In humans, the Pcdh7 gene is located at 4p15 [49] and overlaps a location of frequent chromosomal deletion in hepatocellular carcinomas [50]. In human colon carcinoma cells, the expression of Pcdh7 is altered when cells are treated with the selective cyclo-oxygenase-2 inhibitor NS-398, a nonsteroidal anti-inflammatory drug [51]. This class of drugs can decrease mortality from colorectal cancer. Pcdh7 expression is regulated by the NF-kappaB/IKK inflammatory response pathway in mouse embryonic fibroblasts [52].

Pcdh7 was also called BH-protocadherin because of its prominent expression in the brain and heart of man and mouse [49, 53]. Interestingly, the three alternatively spliced variants have distinct developmental profiles of expression in these tissues [53]. Lower levels of expression were also observed in the human stomach, thyroid gland, spinal cord and placenta [49]. In the brain, Pcdh7 shows the same type of regionally restricted expression (fig. 5A, E, I) as the other δ1-protocadherins [4] and classic cadherins (fig. 5G, H, K, L) [54]. In addition, Pcdh7 is expressed in subpopulations of cells in the trigeminal ganglion (G5 in fig. 5A) and spinal ganglia (SG in fig. 5C), restricted regions of the cochlea (Co in fig. 5A), parts of the head mesenchyme (fig. 5M), the epithelium lining regions of the nasal cavity (nc in fig. 5M), the vomeronasal organ (VNO in fig. 5M), the aorta (Ao in fig. 5C) and specific layers of the hair follicles (hf in fig. 5P).

An ortholog of Pcdh7 in *Xenopus*, termed NF-protocadherin, is expressed in both layers of embryonic ectoderm early in development, and later becomes restricted primarily to the deeper (sensorial) layer. Expression of a dominant negative form of the molecule lacking the extracellular domain results in the formation of large ectodermal blisters [23]. This defect can be rescued by overexpression of NF-protocadherin/Pcdh7 and by overexpression of its cytosolic co-factor, TAF1/Set (see above; fig. 4D) [29]. Blockage of either TAF1/Set or NF-protocadherin/Pcdh7 causes essentially identical ectodermal effects [29]. In the embryonic nervous system of *Xenopus*, expression of NF-protocadherin/Pcdh7 is restricted to subsets of cells in the neural folds and neural tube [23].

Protocadherin-9 (Pcdh9)

Pcdh9 was identified in the human genome during analysis of genes in the 13q14.3-q21.1 region, where it constitutes a linkage group with Pcdh8 [55]. Of the different transcripts [4, 55], the larger ~7.5-kb transcript is expressed predominantly in the human and mouse brain [55]. High levels of expression of a shorter ~5-kb transcript were observed in thymus and testis of man, but not of mouse [55]. In the mouse, a spatially and temporally restricted expression is observed in the nervous system, but it is distinct from that of other δ1-protocadherins (fig. 5B, F, J) [4]. Pcdh9 is also expressed in some cranial ganglia (CoG and vG in fig. 5B), parts of the head mesenchyme, and epithelial regions lining the nasal cavity and the palatal processes (fig. 5N). The differential layer-specific expression of Pcdh7, Pcdh9 and Pcdh11 in the hair follicles is remarkable (fig. 5P-R). Pcdh9 was found to be downregulated in the subplate of the developing neocortex of neurogenin-2 null mutant mice [56].

Protocadherin-11 (Pcdh11)

Pcdh11 was first identified in a database search for genes similar to human Pcdh7 [12]. The gene was mapped to

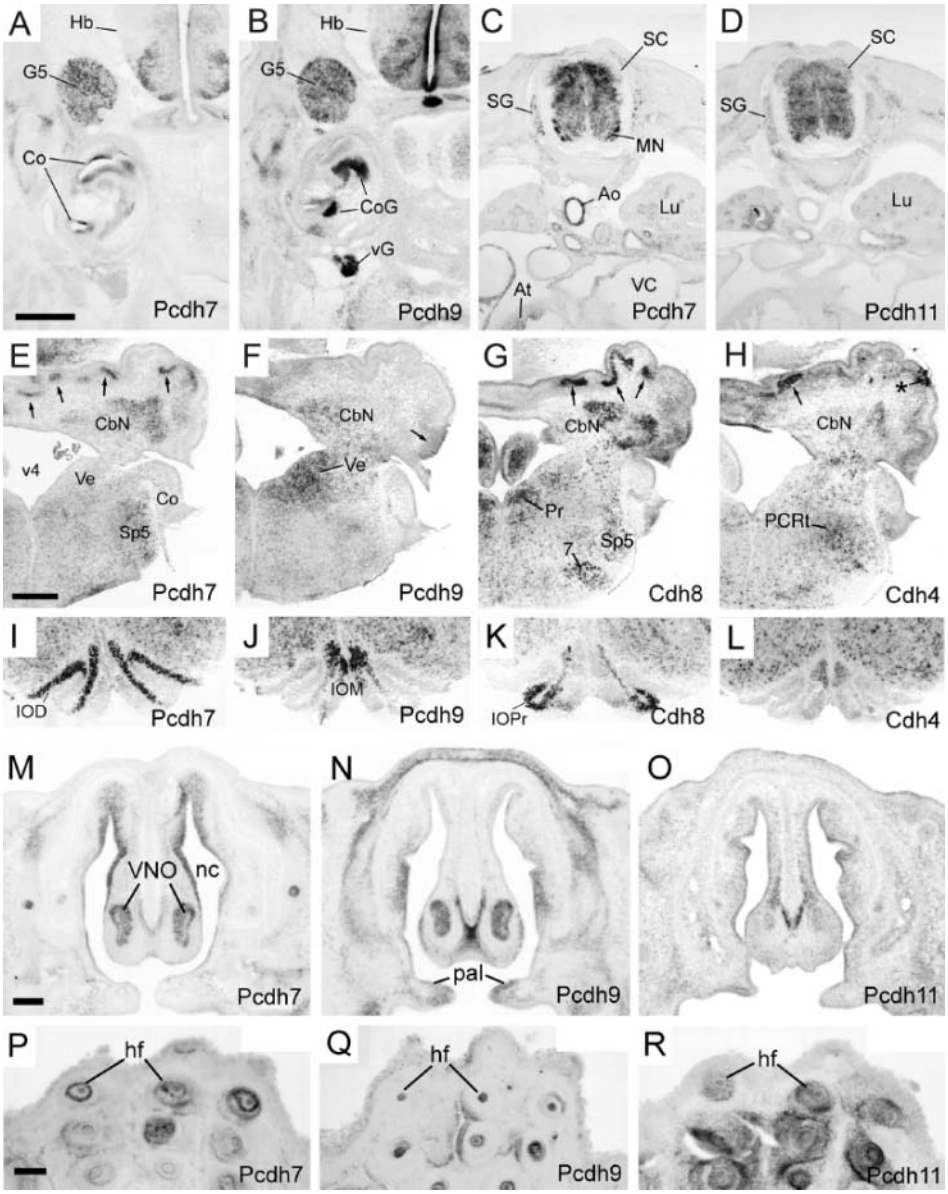

Figure 5. *(A–D)* Transverse section through the head of an E15 mouse embryo (*A, B*), and through the upper thorax of an E15 embryo (*C, D*), hybridized with antisense probes for protocadherin-7 (Pcdh7; *A, C*), protocadherin-9 (Pcdh9; *B*) and protocadherin-11x (Pcdh11; *D*). Note that the trigeminal ganglion (G5) and spinal ganglion (SG) contain subpopulations of Pcdh7-positive neurons. The expression of the protocadherins in the spinal cord (SC) and hindbrain (Hb) is heterogeneous. Specific regions of the cochlear duct (Co) and the wall of the aorta (AO) are Pcdh7 positive. The cochlear ganglion (CoG) expresses Pcdh9. (*E–L*). Coronal sections of P1 mouse brain through the cerebellum (*E–H*) and the brainstem, at the level of the inferior olive *(I–L)*, hybridized with antisense probes for protocadherin-7 (Pcdh7; *E, I*), protocadherin-9 (Pcdh9; *F, J*), type II cadherin-8 (Cdh8; *G, K*) and type I cadherin-4 (Cdh4; *H, L*). The arrows point to parasagittal Purkinje cell domains that express the four cadherins differentially. Note the differential expression by the deep cerebellar nuclei (CbN), each of which receives input from specific cerebellar cortical domains. Moreover, each part of the inferior olive (IO) projects into specific cerebellar cortical domains. The asterisk in H indicates an artifact. (*M–O*) Transverse sections through the E15 nasal cavity (nc) of the mouse, hybridized with antisense probes for Pcdh7 (*M*), Pcdh9 (*N*) and Pcdh11 (*O*). Note that the vomeronasal (Jacobson's) organ (VNO) expresses Pcdh7 and Pcdh9 differentially. Note also the differential staining for all three protocadherins in the mesenchyme of the head and in the epithelium lining the nasal cavity and the palatal processes (pal). (*P–R*). Tangential sections through the E17 whisker pad, hybridized with antisense probes for Pcdh7 (*P*), Pcdh9 (*Q*) and Pcdh11 (*R*). Note the differential expression of the three δ1-protocadherins in the different layers of the hair follicles (hf). Other abbreviations: 7, nucleus of the facial nerve; At, atrium of heart; Co, cochlear nuclei; IOD, dorsal nucleus of the inferior olive; IOM, medial nucleus of the inferior olive; IOPr, principal nucleus of the inferior olive; MN, motor neurons; PCRt, parvicellular reticular nucleus; Pr, prepositus nucleus; Lu, lung; Sp5, spinal trigeminal nucleus; v4, fourth ventricle; VC, vena cava; Ve, vestibular nuclei; vG, visceral ganglion. Scale bars, 0.5 mm (in *A* for *A–D*), 0.5 mm (in *E* for *E–L*), 0.2 mm (in *M* for *M–O*), and 0.1 mm (in *P* for *P–R*).

the XY homology region of the human X chromosome at Xq21.3 and was called protocadherin-X (PcdhX, official gene name PCDH11X). The corresponding gene on the human Y chromosome (PCDH11Y) was identified shortly thereafter and is located at Yp11.2 [13]. The X and Y genes are 98.1% identical at the nucleotide level and 98.3% at the protein level. These differences might nevertheless be functionally significant, and their small magnitude might reflect the relatively short period of time during which the homologs diverged [13].

Pcdh11Y is thought to have emerged as a result of the X-to-Y transposition that occurred during hominid speciation [57]. The gene is present on the Y chromosome in hominids only. Accordingly, only one copy of the Pcdh11 gene was found in the mouse and the pig [58]. The expression of the Pcdh11 gene is relatively widespread in

mouse and pig tissues, whereas – in humans – the gene is primarily expressed in the central nervous system [13, 58]. This finding indicates a possible hominid-specific role in brain development. The human Pcdh11X and Pcdh11Y genes are abundantly expressed also in the fetal human brain [12, 13]. In the mouse, Pcdh11 is expressed in specific brain nuclei as well as in restricted cortical regions and layers of the embryonic and postnatal brain [4]. Some of the Pcdh11-positive brain structures show sexual dimorphisms, such as the globus pallidus, the amygdala and the tuberal hypothalamus [4], consistent with the hypothesis that Pcdh11 contributes to the development of sexual dimorphisms [13]. Interestingly, RT-PCR analysis showed that the various isoforms of the Pcdh11X and Pcdh11Y genes (see also above) are expressed differentially in different regions of the human brain [15]. Expression is also found in restricted regions of developing cartilage in the head (fig. 5O).

Evolutionary changes in the genes transcribed from the X/Y-homology region in humans [59] were proposed to be causally linked to language acquisition and functional brain asymmetry, two hominid-specific features that are absent from the brains of even our closest relatives, chimpanzees. Moreover, it has been speculated that the genetic changes establishing the X/Y-homology region in humans are among the causes of schizophrenia [60]. Two recent studies, however, have not revealed any relation between schizophenia and sequence variations [61] or dosage imbalances [62] of the Pcdh11X/Y genes.

Besides containing a motif that binds to protein phosphatase-1α (see above), the cytoplasmic domains of Pcdh11X and Pcdh11Y contain a serine-rich motif that is similar to the β-catenin binding sequence in E-cadherin [63]. Indeed, β-catenin, which is a well-known proto-oncogene product, was shown to coimmunoprecipitate with protocadherin-PC, which is identical to Pcdh11Y [63]. Interestingly, expression of Pcdh11Y has been found to be induced in human prostate cancer cells during acquisition of apoptosis resistance, androgen-independent growth and neuroendocrine transdifferentiation [63, 64]. Apparently, this protocadherin can be expressed as a cytoplasmic species (fig. 4F), due to use of a start codon 3' of the signal sequence. Experiments with Pcdh11Y-specific small interfering RNAs (siRNAs) in prostate cancer cells revealed that the induction of Pcdh11Y is instrumental in activation of Wnt signaling pathways, neuroendocrine transdifferentiation and survival upon androgen deprivation [63].

δ2-Protocadherins

Protocadherin-8 (Pcdh8)

A gene similar to Pcdh8 was identified in *Xenopus* in an effort to isolate molecules expressed specifically in the dorsal blastopore lip (Spemann organizer) [22]. At a later developmental stage in zebrafish, this gene is expressed in paraxial mesoderm, hence the name paraxial protocadherin (PAPC) [65]. In mouse, the Pcdh8 ortholog is expressed in forming somites [66]. The ortholog in rat, termed arcadlin, was cloned by differential and subtractive cloning techniques carried out to identify mRNAs that were induced in the hippocampus by seizures [19]. These initial reports made it clear that Pcdh8 plays multiple roles in vertebrates, possibly mediated by different isoforms of this protein [16]. In man, the Pcdh8 gene was identified by positional cloning around a CpG-rich island on human chromosomal band 13q14.3 [55].

Evidence from gain- and loss-of-function microinjection experiments in *Xenopus* and zebrafish indicates that PAPC/Pcdh8 is required for dorsal convergence movements during gastrulation [22, 65]. Interestingly, a truncated PAPC/Pcdh8 protein lacking the intracellular domain resulted in stronger adhesion than the full-length cDNA. The opposite effect has been described for a classic type-I cadherin (C-cadherin, a Cdh1 ortholog) [67]. Dominant-negative PAPC/Pcdh8 interferes with gastrulation movements in *Xenopus* embryos and inhibits elongation in explants [22]. PAPC/Pcdh8 expression is lost in nascent mesoderm of Lim-deficient mice and *Xenopus*, and mesoderm in both species is induced but fails to undergo proper gastrulation [68]. In zebrafish, PAPC/Pcdh8 was shown to be a downstream target of *spadetail*, a transcription factor involved in mesodermal morphogenetic movements. In axial mesoderm, which expresses axial protocadherins (AXPC/Pcdh1; see above), the expression of both PAPC/Pcdh8 and *spadetail* is repressed by *floating head*, thereby promoting notochord formation instead of paraxial mesoderm differentiation [65]. In *Xenopus*, PAPC/Pcdh8 can interact with the Frizzled 7 receptor, and modulates the activity of Rho GTPase and c-Jun N-terminal kinase (fig. 4C) [32]. These and other findings [22, 32, 33] indicate that PAPC/Pcdh8 provides a link between regulatory genes in the Spemann's organizer and the execution of cell behaviors during morphogenesis, e. g. by interacting with the planar cell polarity pathway [32, 33].

PAPC/Pcdh8 continues to be expressed in the presomitic paraxial mesoderm of zebrafish and mouse, in which it is required for the establishment and maintenance of segmental boundaries during somite formation [65, 69]. In zebrafish, the expression of PAPC/Pcdh8 in the anterior half of the somitomeres is controlled by the Thylacine 1 and the Notch signaling pathways. Perturbing the activity of these pathways leads to changes in the segmental expression of PAPC/Pcdh8 in the anterior half of the forming somites [70]. Perturbing PAPC/Pcdh8 function, in turn, causes incorrect positioning of anterior and posterior segmental cell markers in zebrafish [70] and in mouse [69]. In mouse, a very similar expression pattern was

found, but mice with Pcdh8 deficiency show no defects in their skeleton and were reported to be viable and fertile [66]. These results indicate that Pcdh8 is redundant with other molecules, possibly related protocadherins.

Expression of Pcdh8 is abundant in the embryonic and adult brain of mouse and man [16, 19, 55, 66]. In mouse brain [16], two isoforms differing in the lengths of their cytoplasmic domains are expressed [5]. Expression of the longer isoform is restricted to neural tissues, whereas the shorter isoform is found also in non-neural tissues, including paraxial mesoderm, developing somites and limb interdigitating mesenchyme [16]. Expression is found in all major divisions of the early embryonic mouse and rat brain and is spatially restricted and developmentally regulated [16, 19].

The role of Pcdh8 in the nervous system was investigated in the rat hippocampus [19], where its expression is dramatically increased after induction of electro-convulsive seizures. Pcdh8 is located at hippocampal synapses. Antibodies against Pcdh8 attenuate basal synaptic transmission and completely inhibit LTP induction in hippocampal slices when applied after a brief incubation in Ca^{2+}-free medium [19].

In the human genome, the Pcdh8 gene maps to a region on chromosome 13 to which schizophrenia is reportedly linked. However, results from one screen for polymorphisms in the gene of schizophrenic individuals suggest no more than a weak contribution of the Pcdh8 gene to susceptibility for this disease [71].

Protocadherin-10 (Pcdh10)

Pcdh10 was initially cloned from cDNA libraries of mouse brain (OL-protocadherin) [20] and human brain (KIAA1400) [72]. The expression of Pcdh10 in developing mouse and chicken brains has been particularly well studied at both the mRNA and protein levels.

Pcdh10 was initially termed OL-protocadherin because high expression levels were observed in specific gray matter areas of the olfactory and limbic systems in the mouse brain [20]. However, in both chicken and mouse, Pcdh10 is also expressed in parts of other functional systems, such as the visual system [73], the cerebellar system [74, 75] and motor neurons of the spinal cord [76]. For example, expression is prominent in neural subcircuits of the developing visual system [73, 77]. In general, the expression of OL-protocadherin resembles that of some classic cadherins [73, 74] in that its expression is restricted to a subset of gray matter structures, such as particular brain nuclei of the hindbrain, midbrain and thalamus. However, the expression pattern of each (proto-)cadherin is distinctly different from those of the other cadherins, though with partial overlap [73]. During development, Pcdh10 is prominently expressed also on specific fiber tracts that connect Pcdh10-positive gray matter structures in several functional systems [73, 76, 77]. Pcdh10 protein is found in synapse-rich regions, e. g. the inner plexiform layer of the retina [73, 77] and the glomeruli of the olfactory bulb [20], suggesting that it might play a role in target recognition and synapse formation, as postulated for many other (proto)cadherins in different species [2, 54].

Other Pcdh10-positive organs include, in adult humans, the heart, kidney, lung and trachea [5], as well as the ovary [72], as determined by PCR and Northern blot analysis, respectively. Little is known about the tissue distribution of Pcdh10 in these organs. In the chicken embryo, mesenchymal cells and the ectoderm of the mandible and limb bud express Pcdh10 [76].

Protocadherin-17 (Pcdh17)

Pcdh17 (also called protocadherin-68) genes have been isolated from several species, including human, rhesus monkey, cow, mouse, rat, chicken and zebrafish. Little is known about the expression or function of this gene.

Protocadherin-18 and -19 (Pcdh18 and Pcdh19)

Pcdh18 (KIAA1562) and Pcdh19 (KIAA 1313) were identified by a database search [5] for molecules that contain the CM2 motif, which is highly conserved in δ-protocadherin genes of different vertebrate species (see above). Both Pcdh18 and Pcdh19 are expressed in the brain and in several other organs, including heart, kidney, lung and trachea [5, 40].

Using a yeast two-hybrid system, Pcdh18 was shown to interact with mouse disabled-1 (Dab1; see above; fig. 4B) [40].

Acknowledgements. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Re 616/4-4 to C.R.), the IZKF Jena (TP 1.16 to C.R.), the VIB (Vlaams Interuniversitair Instituut voor Biotechnologie to F.v.R.) and the FWO-Flanders (National Fund for Scientific Research to F.v.R). We thank Katrien Staes for technical assistance and constructive discussions and Dr. Amin Bredan for editorial assistance.

1 Nollet F., Kools P. and van Roy F. (2000) Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J. Mol. Biol. **299:** 551–572
2 Hirano S., Suzuki S. T. and Redies C. (2003) The cadherin superfamily in neural development: diversity, function and interaction with other molecules. Front. Biosci. **8:** d306–356
3 Frank M. and Kemler R. (2002) Protocadherins. Curr. Opin. Cell Biol. **14:** 557–562.
4 Vanhalst K., Kools P., Staes K., van Roy F. and Redies C. (2005) δ-Protocadherins: a gene family expressed differentially in the mouse brain. Cell. Mol. Life Sci. **62:** 1247–1259
5 Wolverton T. and Lalande M. (2001) Identification and characterization of three members of a novel subclass of protocadherins. Genomics **76:** 66–72
6 Vanhalst K., Kools P., Vanden Eynde E. and van Roy F. (2001) The human and murine protocadherin-β one-exon gene families show high evolutionary conservation, despite the difference in gene number. FEBS Lett. **495:** 120–125
7 Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F. and Higgins D. G. (1997) The clustalX windows interface: flexible

strategies for multiple sequence. Nucl. Acids Res. **24:** 4876–4882
8 Page R. D. M. (1996) Treeview: an application to display phylogenetic trees on personal computers. Computer Applic. Biosc. **12:** 357–358
9 Wu Q. and Maniatis T. (1999) A striking organization of a large family of human neural cadherin-like cell adhesion genes. Cell **97:** 779–790
10 Nakajima D., Nakayama M., Kikuno R., Hirosawa M., Nagase T. and Ohara O. (2001) Identification of three novel non-classical cadherin genes through comprehensive analysis of large cDNAs. Brain Res. Mol. Brain Res. **94:** 85–95
11 Hong J. C., Ivanov N. V., Hodor P., Xia M., Wei N., Blevins R. et al. (2004) Identification of new human cadherin genes using a combination of protein motif search and gene finding methods. J. Mol. Biol. **337:** 307–317
12 Yoshida K. and Sugano S. (1999) Identification of a novel protocadherin gene (PCDH11) on the human XY homology region in Xq21.3. Genomics **62:** 540–543
13 Blanco P., Sargent C. A., Boucher C. A., Mitchell M. and Affara N. A. (2000) Conservation of PCDHX in mammals; expression of human X/Y genes predominantly in brain. Mamm. Genome **11:** 906–914
14 Sugino H., Hamada S., Yasuda R., Tuji A., Matsuda Y., Fujita M. et al. (2000) Genomic organization of the family of CNR cadherin genes in mice and humans. Genomics **63:** 75–87
15 Blanco-Arias P., Sargent C. and Affara N. (2004) Protocadherin X (PCDHX) and Y (PCDHY) genes; multiple mRNA isoforms encoding variant signal peptides and cytoplasmic domains. Mamm. Genome **15:** 41–52
16 Makarenkova H., Sugiura H., Yamagata K. and Owens G. (2005) Alternatively spliced variants of protocadherin 8 exhibit distinct patterns of expression during mouse development. Biochim. Biophys. Acta **1681:** 150–156
17 Sano K., Tanihara H., Heimark R. L., Obata S., Davidson M., St. John T. et al. (1993) Protocadherins: a large family of cadherin-mediated molecules in central nervous system. EMBO J. **12:** 2249–2256
18 Yoshida K. (2003) Fibroblast cell shape and adhesion in vitro is altered by overexpression of the 7a and 7b isoforms of protocadherin 7, but not the 7c isoform. Cell. Mol. Biol. Lett. **8:** 735–741
19 Yamagata K., Andreasson K. I., Sugiura H., Maru E., Dominique M., Irie Y. et al. (1999) Arcadlin is a neural activity-regulated cadherin involved in long term potentiation. J. Biol. Chem. **274:** 19473–19479
20 Hirano S., Yan Q. and Suzuki S. T. (1999) Expression of a novel protocadherin, OL-protocadherin, in a subset of functional systems of the developing mouse brain. J. Neurosci. **19:** 995–1005
21 Kuroda H., Inui M., Sugimoto K., Hayata T. and Asashima M. (2002) Axial protocadherin is a mediator of prenotochord cell sorting in Xenopus. Dev. Biol. **244:** 267–277
22 Kim S. H., Yamamoto A., Bouwmeester T., Agius E. and Robertis E. M. (1998) The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during Xenopus gastrulation. Development **125:** 4681–4690
23 Bradley R. S., Espeseth A. and Kintner C. (1998) NF-protocadherin, a novel member of the cadherin superfamily, is required for Xenopus ectodermal differentiation. Curr. Biol. **8:** 325–334
24 Egloff M. P., Johnson D. F., Moorhead G., Cohen P. T., Cohen P. and Barford D. (1997) Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. EMBO J. **16:** 1876–1887
25 Yoshida K., Watanabe M., Kato H., Dutta A. and Sugano S. (1999) BH-protocadherin-c, a member of the cadherin superfamily, interacts with protein phosphatase 1 alpha through its intracellular domain. FEBS Lett. **460:** 93–98
26 da Cruz e Silva E. F., Fox C. A., Ouimet C. C., Gustafson E., Watson S. J. and Greengard P. (1995) Differential expression of protein phosphatase 1 isoforms in mammalian brain. J. Neurosci. **15:** 3375–3389
27 Ouimet C. C., da Cruz e Silva E. F. and Greengard P. (1995) The alpha and gamma 1 isoforms of protein phosphatase 1 are highly and specifically concentrated in dendritic spines. Proc. Natl. Acad. Sci. USA **92:** 3396–3400
28 Terry-Lorenzo R. T., Carmody L. C., Voltz J. W., Connor J. H., Li S., Smith F. D. et al. (2002) The neuronal actin-binding proteins, neurabin I and neurabin II, recruit specific isoforms of protein phosphatase-1 catalytic subunits. J. Biol. Chem. **277:** 27716–27724
29 Heggem M. and Bradley R. (2003) The cytoplasmic domain of *Xenopus* NF-protocadherin interacts with TAF1/set. Dev. Cell **4:** 419–429
30 Qu D., Li Q., Lim H. Y., Cheung N. S., Li R., Wang J. H. et al. (2002) The protein SET binds the neuronal Cdk5 activator p35nck5a and modulates Cdk5/p35nck5a activity. J. Biol. Chem. **277:** 7324–7332
31 Cervoni N., Detich N., Seo S. B., Chakravarti D. and Szyf M. (2002) The oncoprotein Set/TAF-1beta, an inhibitor of histone acetyltransferase, inhibits active demethylation of DNA, integrating DNA methylation and transcriptional silencing. J. Biol. Chem. **277:** 25026–25031
32 Medina A., Swain R., Kuerner K. and Steinbeisser H. (2004) Xenopus paraxial protocadherin has signaling functions and is involved in tissue separation. EMBO J. **23:** 3249–3258
33 Unterseher F., Hefele J., Giehl K., De Robertis E., Wedlich D. and Schambony A. (2004) Paraxial protocadherin coordinates cell polarity during convergent extension via Rho A and JNK. EMBO J. **23:** 3259–3269
34 Formstone C. J. and Mason I. (2005) Combinatorial activity of Flamingo proteins directs convergence and extension within the early zebrafish embryo via the planar cell polarity pathway. Dev. Biol. **282:** 320–335
35 Tissir F., Bar I., Jossin Y. and Goffinet A. M. (2005) Protocadherin Celsr3 is crucial in axonal tract development. Nat. Neurosci. **8:** 451–457
36 Kohmura N., Senzaki K., Hamada S., Kai N., Yasuda R., Watanabe N. et al. (1998) Diversity revealed by a novel family of cadherins expressed in neurons at a synaptic complex. Neuron **20:** 1137–1151
37 Jossin Y., Ignatova N., Hiesberger T., Herz J., Lambert de Rouvroit C. and Goffinet A. M. (2004) The central fragment of Reelin, generated by proteolytic processing in vivo, is critical to its function during cortical plate development. J. Neurosci. **24:** 514–521
38 Bar I., Lambert de Rouvroit C. and Goffinet A. M. (2000) The evolution of cortical development. An hypothesis based on the role of the Reelin signaling pathway. Trends Neurosci. **23:** 633–638
39 Senzaki K., Ogawa M. and Yagi T. (1999) Proteins of the CNR family are multiple receptors for Reelin. Cell **99:** 635–647
40 Homayouni R., Rice D. S. and Curran T. (2001) Disabled-1 interacts with a novel developmentally regulated protocadherin. Biochem. Biophys. Res. Comm. **289:** 539–547
41 Gayet O., Labella V., Henderson C. E. and Kallenbach S. (2004) The b1 isoform of protocadherin-gamma (Pcdhgamma) interacts with the microtubule-destabilizing protein SCG10. FEBS Lett. **578:** 175–179
42 Hambsch B., Grinevich V., Seeburg P. H. and Schwarz M. K. (2005) γ-Protocadherins, presenilin-mediated release of C-terminal fragment promotes locus expression. J. Biol. Chem. **280:** 15888–15897
43 Haas I. G., Frank M., Veron N. and Kemler R. (2005) Presenilin-dependent processing and nuclear function of gamma-protocadherins. J. Biol. Chem. **280:** 9313–9319
44 Magg T., Schreiner D., Solis G. P., Bade E. G. and Hofer H. W. (2005) Processing of the human protocadherin Fat1 and translocation of its cytoplasmic domain to the nucleus. Exp. Cell Res. **307:** 100–108

45 Obata S., Sago H., Mori N., Rochelle J. M., Seldin M. F., Davidson M. et al. (1995) Protocadherin Pcdh2 shows properties similar to, but distinct from, those of classical cadherins. J. Cell Sci. **108:** 3765–3773
46 Favre C. J., Mancuso M., Maas K., McLean J. W., Baluk P. and McDonald D. M. (2003) Expression of genes involved in vascular development and angiogenesis in endothelial cells of adult lung. Am. J. Physiol. Heart Circ. Physiol. **285:** H1917–1938
47 Maxwell S. A. and Davis G. E. (2000) Biological and molecular characterization of an ECV-304-derived cell line resistant to p53-mediated apoptosis. Apoptosis **5:** 277–290
48 Fletcher S. T., Baker V. A., Fentem J. H., Basketter D. A. and Kelsell D. P. (2001) Gene expression analysis of EpiDerm following exposure to SLS using cDNA microarrays. Toxicol. In Vitro **15:** 393–398
49 Yoshida K., Yoshitomo-Nakagawa K., Seki N., Sasaki M. and Sugano S. (1998) Cloning, expression analysis and chromosomal localization of BH-protocadherin (PCDH7), a novel member of the cadherin superfamily. Genomics **49:** 458–461
50 Zondervan P. E., Wink J., Alers J. C., Ijzermans I. N., Schalm S. W., de Man R. A. et al. (2000) Molecular cytogenetic evaluation of virus-associated and non-viral hepatocellular carcinoma: analysis of 26 carcinomas and 12 concurrent dysplasias. J. Pathol. **192:** 207–215
51 Zhang Z. and DuBois R. N. (2001) Detection of differentially expressed genes in human colon carcinoma cells treated with a selective COX-2 inhibitor. Oncogene **20:** 4450–4456
52 Li X., Massa P. E., Hanidu A., Peet G. W., Aro P., Savitt A. et al. (2002) IKKalpha, IKKbeta and NEMO/IKKgamma are each required for the NF-kappa B-mediated inflammatory response program. J. Biol. Chem. **277:** 45129–45140
53 Yoshida K., Hida M., Watanabe M., Yamaguchi R., Tateyama S. and Sugano S. (1999) cDNA cloning and chromosomal mapping of mouse BH-protocadherin. DNA Seq. **10:** 43–47
54 Redies C. (2000) Cadherins in the central nervous system. Prog. Neurobiol. **61:** 611–648
55 Strehl S., Glatt K., Liu Q. M., Glatt H. and Lalande M. (1998) Characterization of two novel protocadherins (PCDH8 and PCDH9) localized on human chromosome 13 and mouse chromosome 14. Genomics **53:** 81–89
56 Mattar P., Britz O., Johannes C., Nieto M., Ma L., Rebeyka A. et al. (2004) A screen for downstream effectors of Neurogenin2 in the embryonic neocortex. Dev. Biol. **273:** 373–389
57 Page D. C., Harper M. E., Love J. and Botstein D. (1984) Occurrence of a transposition from the X-chromosome long arm to the Y-chromosome short arm during human evolution. Nature **311:** 119–123
58 Blanco-Arias P., Sargent C. and Affara N. (2004) A comparative analysis of the pig, mouse and human PCDHX genes. Mamm. Genome **15:** 296–306
59 Lopes A. M., Calafell F. and Amorim A. (2004) Microsatellite variation and evolutionary history of PCDHX/Y gene pair within the Xq21.3/Yp11.2 hominid-specific homology block. Mol. Biol. Evol. **21:** 2092–2101
60 Crow T. J. (2002) Handedness, language lateralisation and anatomical asymmetry: relevance of protocadherin XY to hominid speciation and the aetiology of psychosis. Point of view. Brit. J. Psychiat. **181:** 295–297.
61 Giouzeli M., Williams N., Lonie L., DeLisi L. and Crow T. (2004) ProtocadherinX/Y, a candidate gene-pair for schizophrenia and schizoaffective disorder: a DHPLC investigation of genomic sequence. Am. J. Med. Genet. **129:** 1–9
62 Ross N. L., Mavrogiannis L. A., Sargent C. A., Knight S. J., Wadekar R., DeLisi L. E. et al. (2003) Quantitation of X-Y homologous genes in patients with schizophrenia by multiplex polymerase chain reaction. Psych. Genetics **13:** 115–119
63 Ji J., Chen X., Leung S. Y., Chi J. T., Chu K. M., Yuen S. T. et al. (2002) Comprehensive analysis of the gene expression profiles in human gastric cancer cell lines. Oncogene **21:** 6549–6556
64 Yang X., Chen M. W., Terry S., Vacherot F., Chopin D. K., Bemis D. L. et al. (2005) A human- and male-specific protocadherin that acts through the wnt signaling pathway to induce neuroendocrine transdifferentiation of prostate cancer cells. Cancer Res. **65:** 5263–5271
65 Yamamoto A., Amacher S. L., Kim S. H., Geissert D., Kimmel C. B. and De Robertis E. M. (1998) Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. Development **125:** 3389–3397
66 Yamamoto A., Kemp C., Bachiller D., Geissert D. and De Robertis E. M. (2000) Mouse paraxial protocadherin is expressed in trunk mesoderm and is not essential for mouse development. Genesis **27:** 49–57
67 Lee C. H. and Gumbiner B. M. (1995) Disruption of gastrulation movements in Xenopus by a dominant-negative mutant for C-cadherin. Dev. Biol. **171:** 363–373
68 Hukriede N. A., Tsang T. E., Habas R., Khoo P. L., Steiner K., Weeks D. L. et al. (2003) Conserved requirement of Lim1 function for cell movements during gastrulation. Dev. Cell **4:** 83–94
69 Rhee J., Takahashi Y., Saga Y., Wilson-Rawls J. and Rawls A. (2003) The protocadherin papc is involved in the organization of the epithelium along the segmental border during mouse somitogenesis. Dev. Biol. **254:** 248–261
70 Kim S. H., Jen W. C., De Robertis E. M. and Kintner C. (2000) The protocadherin PAPC establishes segmental boundaries during somitogenesis in xenopus embryos. Current Biol. **10:** 821–830
71 Bray N. J., Kirov G., Owen R. J., Jacobsen N. J., Georgieva L., Williams H. J. et al. (2002) Screening the human protocadherin 8 (PCDH8) gene in schizophrenia. Genes Brain Behav. **1:** 187–191
72 Nagase T., Kikuno R., Ishikawa K. I., Hirosawa M. and Ohara O. (2000) Prediction of the coding sequences of unidentified human genes. XVI. The complete sequences of 150 new cDNA clones from brain which code for large proteins in vitro. DNA Res. **7:** 65–73
73 Müller K., Hirano S., Puelles L. and Redies C. (2004) OL-protocadherin expression in the visual system of the chicken embryo. J. Comp. Neurol. **470:** 240–255
74 Luckner R., Obst-Pernberg K., Hirano S., Suzuki S. T. and Redies C. (2001) Granule cell raphes in the developing mouse cerebellum. Cell Tissue Res. **303:** 159–172
75 Hirano S., Yan Q. and Suzuki S. T. (1999) Expression of a novel protocadherin, OL-protocadherin, in a subset of functional systems of the developing mouse brain. J. Neurosci. **19:** 995–1005
76 Nakao S., Uemura M., Aoki E., Suzuki S. T., Takeichi M. and Hirano S. (2005) Distribution of OL-protocadherin in axon fibers in the developing chick nervous system. Brain Res. Mol. Brain Res. **134:** 294–308
77 Aoki E., Kimura R., Suzuki S. T. and Hirano S. (2003) Distribution of OL-protocadherin protein in correlation with specific neural compartments and local circuits in the postnatal mouse brain. Neuroscience **117:** 593–614
78 Chen M. W., Vacherot F., De La Taille A., Gil-Diez-De-Medina S., Shen R., Friedman R. A. et al. (2002) The emergence of protocadherin-PC expression during the acquisition of apoptosis-resistance by prostate cancer cells. Oncogene **21:** 7861–7871
79 Nagase T., Kikuno R., Nakayama M., Hirosawa M. and Ohara O. (2000) Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res. **7:** 273–281