Review

Clustered protocadherin family

Takeshi Yagi*

KOKORO-Biology Group, Laboratories for Integrated Biology, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan

The brain is a complex system composed of enormous numbers of differentiated neurons, and brain structure and function differs among vertebrates. To examine the molecular mechanisms underlying brain structure and function, it is important to identify the molecules involved in generating neural diversity and organization. The clustered protocadherin (Pcdh) family is the largest subgroup of the diverse cadherin superfamily. The clustered Pcdh proteins are predominantly expressed in the brain and their gene structures in vertebrates are diversified. In mammals, the clustered Pcdh family consists of three gene clusters: Pcdh-α, Pcdh-β, and Pcdh-γ. During brain development, this family is upregulated by neuronal differentiation, and Pcdh-α is then dramatically downregulated by myelination. Clustered Pcdh expression continues in the olfactory bulb, hippocampus, and cerebellum until adulthood. Structural analysis of the first cadherin domain of the Pcdh-α protein revealed it lacks the features that classical cadherins require for homophilic adhesiveness, but it contains Pcdh-specific loop structures. In Pcdh-α, an RGD motif on a specific loop structure binds β1-integrin.

For gene expression, the gene clusters are regulated by multiple promoters and alternative cis splicing. At the single-cell level, several dozen Pcdh-α and -γ mRNA are regulated monoallelically, resulting in the combinatorial expression of distinct variable exons. The Pcdh-α and Pcdh-γ proteins also form oligomers, further increasing the molecular diversity at the cell surface. Thus, the unique features of the clustered Pcdh family may provide the molecular basis for generating individual cellular diversity and the complex neural circuitry of the brain.

Key words: brain, CNR, evolution, integrin, neuronal diversity, Reelin.

Introduction

The vertebrate brain differs in its structural organization and functioning across species. It contains enormous numbers of differentiated individual neurons that form complex neural networks. To understand the biological mechanisms of the brain, we need to learn how neuronal diversity is generated and the neural networks organized, on the molecular level. Cell-adhesion molecules are known to be involved in many biological events, including cell recognition, cell signaling, and the formation of neural networks. Cadherins were originally characterized as calcium-dependent cell adhesion molecules, and are identified by their cadherin sequence motifs, which contain about 110 amino acids (Takeichi 1988). The recent sequencing of several vertebrate genomes has revealed the impressive diversity of the cadherin superfamily, in which more than 100 different cadherin-related genes have been identified (Yagi & Takeichi 2000). The protocadherin (Pcdh) family is a large subgroup within the cadherin superfamily in vertebrates, and the Pcdh genes are predominantly expressed in the brain. The Pcdh family is divided into two groups based on their genomic structure: the clustered and nonclustered Pcdh families (Morishita & Yagi 2007). With over 50 members, the clustered Pcdh family represents the largest subgroup in the cadherin superfamily (Kohmura et al. 1998; Wu & Maniatis 1999). In mammals, the clustered Pcdh family consists of the Pcdh-α, Pcdh-β, and Pcdh-γ genes (Hirayama & Yagi 2006). These gene clusters are generally organized into a variable region containing a tandem array of variable exons and a constant region containing one set of constant exons (Fig. 1).
Molecular evolution

Clustered Pcdh genes have been identified in several vertebrate species: human, chimpanzee, rat, mouse, chicken, zebrafish, fugu, and coelacanth (Wu & Maniatis 1999; Sugino et al. 2000; Noonan et al. 2004a,b; Sugino et al. 2004; Tada et al. 2004; Yanase et al. 2004; Wu 2005; Yu et al. 2007; Fig. 2). Invertebrate species contain cadherin-related genes, but not clustered Pcdh genes. In vertebrates, the clustered Pcdh genes are predominantly expressed in the brain. Therefore, the Pcdh gene clusters were generated in the genome of a common vertebrate ancestor and diversified during brain evolution. Phylogenetic analyses of the Pcdh gene clusters demonstrated that the constant region of the Pcdh-α and Pcdh-γ clusters is highly conserved among vertebrates (Fig. 2). Teleost fishes have two unlinked clustered Pcdh loci. Zebrafish has one set of the Pcdh-α and Pcdh-γ clusters in each of these loci (Noonan et al. 2004b; Tada et al. 2004). Fugu possesses one set of the Pcdh-α and Pcdh-γ clusters in one locus, and only the Pcdh-α cluster in the other (Yu et al. 2007). The constant regions of the Pcdh-α and Pcdh-γ clusters in teleosts are also highly conserved (Fig. 2).

The Pcdh variable exons are orthologous among human, rat, and mouse (Sugino et al. 2000; Noonan et al. 2004a; Yanase et al. 2004; Wu 2005). In those of the chicken, zebrafish, fugu, and coelacanth, however, such orthologous relationships are not found (Noonan et al. 2004a,b; Sugino et al. 2004; Tada et al. 2004; Yu et al. 2007). In addition, unusual splicing forms that are not seen in mammals are generated from the more variable region of the Pcdh-α genes in zebrafish. Nevertheless, the putative promoter elements upstream of the variable exons are highly conserved across species (Tada et al. 2004). Analysis of single nucleotide polymorphisms (SNP) of the Pcdh-α variable exons among wild-derived and laboratory mouse strains demonstrated that synonymous (i.e., no change in the amino acid) SNP are concentrated in the first and fifth extracellular cadherin (EC) domains within each variable exon (Taguchi et al. 2005). These domains have high nucleotide homology among the Pcdh-α paralogues, suggesting that gene conversion events in the homologous domains are related to the generation of SNP. A phylogenetic analysis revealed gene conversion events in the EC1 and EC5 domains. Interestingly, the guanine/cytosine (GC) content of the third nucleotide in each codon is higher.

Fig. 1. Genomic organization of the mouse clustered Pcdh genes. Three clusters are present in chromosome 18: Pcdh-α (α), Pcdh-β (β), and Pcdh-γ (γ). The gene clusters include variable and constant regions, although the Pcdh-β cluster does not have a constant region. The Pcdh-α C1 and -α2 variable exons exhibit high homology with Pcdh-γ C3, -γ4, and -γ5. The small black boxes indicate identified DNase I hypersensitive enhancer sites (HS7 and HS5-1) in the Pcdh-α cluster. A conserved sequence element (CSE) is almost always present in the promoter region upstream of each variable exon. The CSE sequence, however, is not conserved in the Pcdh-αC2 promoter region. The mature mRNA of Pcdh-α is produced from one of these variable exons and a set of three constant region exons by cis-splicing. Each variable exon encodes a single extracellular, transmembrane (TM), and cytoplasmic region, and the Pcdh-α and -γ constant exons encode the cytoplasmic tails. All the clustered Pcdh proteins consist of a signal peptide with six extracellular cadherin (EC) domains in the extracellular region. In addition, there is a Cys-(X)5-Cys (C-X5-C) motif in the EC1 domain of all Pcdh proteins, and an RGD motif is well conserved in the mammalian Pcdh-α proteins. The ribbon diagram of the representative NMR structure of the EC1 domain of Pcdh-α4 Morishita et al. (2006).
in the EC1 and EC5 domains, although a bias for GC to adenine/thymine (AT) substitutions is found in all the codon positions (Taguchi et al. 2005). Paralogous relationships as a result of gene conversion have been demonstrated in several multigene families that show increased GC content at the third codon position (Drouin et al. 1999). Thus, there is evidence for gene conversion events in the Pcdh clusters (Noonan et al. 2004b; Miki et al. 2005; Taguchi et al. 2005). In addition, findings indicate that lineage-specific duplication of variable exons, region-restricted gene conversion within species, and adaptive variation have all driven the evolution of the clustered Pcdh genes (Noonan et al. 2004b).

In humans, the Pcdh gene cluster is located at 5q31 and contains many SNP (Kirov et al. 2003; Noonan et al. 2003; Miki et al. 2005). The polymorphisms in the Pcdh-α gene cluster include amino acid exchanges, extensive linkage disequilibrium, and deletions (Noonan et al. 2003; Miki et al. 2005). This molecular diversity in the human population is compatible with the clustered Pcdh genes being candidates for responsible genes of psychiatric diseases as well as for determining functional characteristics of the human brain.

**Protein structure and cell adhesion**

Protein structural analysis has provided significant insight into the molecular basis for the cell adhesion of classical cadherins (Patel et al. 2003). The homophilic adhesive binding region of classical cadherin is located in the EC1 domain (Boggon et al. 2002; Morishita et al. 2006). To determine whether the clustered Pcdh proteins share these characteristics, the solution protein structure of the EC1 domain of Pcdh-α4 was determined by nuclear magnetic resonance (NMR) (Umitsu et al. 2005; Morishita et al. 2006). The topology, a β-sandwich-like structure composed of two packed β sheets, resembles the EC1 domain of classical cadherins, although the sequence similarity between the EC1 domains of Pcdh-α4 and of
classical cadherins is low (30% maximum). However, the interconnecting loops of the Pcdhs are divergent from those of the classical cadherins in length and biochemical properties.

The Pcdh-α4 EC1 domain does not have a tryptophan (W2) or a deep hydrophobic pocket, which are essential for the homophilic adhesive property of the classical cadherins (Boggon et al. 2002; Morishita et al. 2006). The deep hydrophobic pocket is highly conserved among the classical, type II, and desmosomal cadherins (Patel et al. 2003). Although the Pcdh-α4 EC1 domain has a hydrophobic cluster in the corresponding region, the pocket is not as deep as in classical cadherins. The lack of W2 and a deep hydrophobic pocket suggests that the Pcdhs do not have the homophilic adhesion property of the classical cadherins. Consistent with these structural characteristics, a biochemical analysis of the Pcdh-α4 protein by a protein-coated bead aggregation assay showed little trans-homophilic adhesion activity compared with that of N-cadherin (Morishita et al. 2006). On the other hand, it is reported that Pcdh proteins form cis-homodimers (Pcdh-α and Pcdh-α (Triana-Baltzer & Blank 2006), or Pcdh-γ and Pcdh-γ (Hambsch et al. 2005)), and cis-heterodimers (Pcdh-α and Pcdh-γ (Murata et al. 2004)) on the same cell surface. It is possible that Pcdh proteins possess trans-homophilic adhesive activity after they form Pcdh complexes with other Pcdh proteins (see Fig. 3).

The structural characterization has also been performed for the loop regions of the Pcdh EC1 domain, including the Pcdh-specific disulfide-bonded Cys-(X)5-Cys motif, and the RGD (Arg-Gly-Asp) motif (Fig. 1). Interestingly, the Cys-(X)5-Cys motif is well conserved among the Pcdhs, but not in classical cadherins (Morishita & Yagi 2007). Structural analysis revealed that the Cys-(X)5-Cys motif is exposed to solvent as a loop, with the two cysteines forming a disulfide bond (Morishita et al. 2006). The disulfide-bonded loop of a Cys-(X)5-Cys motif provides a protein–protein interaction site for viral glycoproteins (Poubmbourios et al. 2003), suggesting that the Cys-(X)5-Cys motif specific to the Pcdh family contributes to its function by acting as a novel adhesion site. The RGD motif in the EC1 of Pcdh-α4 is also in a loop region that is exposed to solvent (Fig. 1). The RGD motif is a well-known binding motif for integrin (Ruoslahti & Pierschbacher 1987). Consistent with the structural characteristics of the Pcdh-α4 protein, heterophilic cell adhesion activity was observed between Pcdh-α4 and β1 integrin in a cell aggregation assay with HEK293T cells (Mutoh et al. 2004). The activation of integrin is necessary for cells to bind immobilized Pcdh-α4 EC1 fragments (Morishita et al. 2006). The RGD motif is conserved in mammalian Pcdh-α family members, although it is absent from the Pcdh-α proteins of other vertebrates. Therefore, the interaction between the Pcdh-α family and β1 integrin may determine certain neural features that are characteristic of mammals.

Pcdh proteins are reported to be regulated by proteolysis. The Pcdh-α and Pcdh-γ proteins are specifically cleaved by ADAM10 and presenilin (Haas et al. 2005; Hambsch et al. 2005; Reiss et al. 2006; Bonn et al. 2007), and this proteolysis modulates cell adhesion and Pcdh-α and Pcdh-γ cis-heterodimer complex formation (Fig. 3). In addition, the further proteolysis of Pcdh-α and Pcdh-γ generates cytoplasmic fragments, which translocate into the nucleus from the plasma membrane (Haas et al. 2005; Hambsch et al. 2005; Bonn et al. 2007). Overexpression of the Pcdh-γ cytoplasmic domain can transactivate several Pcdh-γ promoters, but not the Pcdh-α promoters, in a manner that does not depend on the conserved element (CSE) (Hambsch et al. 2005). Therefore the proteolysis of Pcdh-γ proteins in cellular membrane might induce transactivation of the Pcdh-γ promoters. The down-regulation of Pcdh-γ proteins in mutant mice lacking the cytoplasmic tail of Pcdh-γ is thought to be caused by the loss of this feedback reaction. However, the truncated transcripts from the knock-out mice, which possess a frame-shift STOP codon resulting in a skipped third exon, are degraded by the nonsense-mediated mRNA decay (NMD) reaction (Culbertson 1999). Thus, the biochemical mechanisms behind the feedback reaction in the Pcdh-γ gene cluster are not yet clear.

**Pcdh protein interactions**

During neocortical development, cortical plate neurons express Pcdh-α mRNA, and Pcdh-α proteins are found in the marginal zone, subplate, and intermediate zone (Senzaki et al. 1999; Morishita et al. 2004b). Cellular interactions between the cortical neurons and the Cajal-Retzius cells in the marginal zone are important for the layering and positioning of neocortical neurons. Reelin expressed in the Cajal-Retzius cells is a key molecule for appropriate layering (D’Arcangelo & Curran 1998). We showed that the RGD motif of Pcdh-α4 protein binds to the first Reelin repeat in the N-terminus of Reelin (Senzaki et al. 1999). We showed this using detergent-solubilized crude extracts from the transfected cells, because the Reelin-alkaline phosphatase (AP) fusion proteins used in the binding experiments were not secreted from the transfected HEK293 cells. However, Jossin et al. reported that the secreted form of Reelin from transfected cells binds to very-low-density lipoprotein.
receptor (VLDLR) and apolipoprotein-E receptor type 2 (ApoER2), but not to the Pcdh-a4 protein (Jossin et al. 2004). Reelin binds to VLDLR and ApoER2 via Reelin repeats 3–6, in the middle of the Reelin protein. The authors speculated that the Reelin-AP fusion protein used in our previous study contained misfolded Reelin-AP fusion proteins, and that the misfolded proteins bound the Pcdh-a4 protein nonspecifically (Jossin et al. 2004).

On the other hand, as described above, our recent studies demonstrated that the Pcdh-a4 protein interacts with β1 integrin expressed in HEK293 cells (Mutoh et al. 2004; Morishita et al. 2006). The RGD motif of Pcdh-a4 is required for its binding to β1 integrin. This RGD motif is the same site as we found to bind the Reelin-AP fusion protein (Senzaki et al. 1999).

In addition, Dulabon et al. (2000) demonstrated that Reelin binds to the α3β1 integrin. The α3β1 integrin binds directly to a secreted form of Reelin, and the N-terminal region of Reelin is required for this binding (Schmid et al. 2005). The N-terminal region of Reelin, which contains the first Reelin repeat, is the region we identified as binding the Pcdh-a4 protein (Senzaki et al. 1999).

Thus, both the RGD motif of Pcdh-a4 and the N-terminal region of Reelin-AP can bind integrins, sug-
sisting that the Pcdh-α4 protein might bind the secreted form of Reelin indirectly, through integrins. Therefore, in our experiments, the Pcdh-α4 protein may have bound to the integrin/Reelin protein complex through its RGD motif in the protein extracts from the transfected HEK293 cells. It is also possible that Pcdh-α4 protein does not bind directly to the secreted full-length Reelin protein (as shown in Jossin et al. 2004), but rather to a protein complex of integrin and Reelin. However, the function of the interaction between Pcdh-α4 and integrin (or Reelin) in vivo is not yet known.

Subcellularly, fractionation experiments showed that both the Pcdh-α and Pcdh-γ proteins are enriched in the post-synaptic density (PSD) fraction, and double-label immunostaining showed that their signals partially overlap. It is reported that overexpressed Pcdh-α proteins are barely expressed on the cell surface (Mutoh et al. 2004). Interestingly, when Pcdh-α and Pcdh-γ are coexpressed in HEK293T cells, there is a more than fivefold increase in the cell-surface expression of Pcdh-α (Murata et al. 2004). These results suggest that Pcdh-α and Pcdh-γ form a hetero-protein complex (Fig. 3). The Pcdh-α and Pcdh-γ interaction was confirmed by co-immunoprecipitation studies using extracts of mouse brain and neuroblastoma. This combinatorial expression of the Pcdh-α and Pcdh-γ proteins would allow individual neurons to express more diverse surface molecules, perhaps establishing their neuronal cell identity and serving as molecular signals for neural network formation. However, using the Pcdh-γ targeting mice lacking the first constant exon, no marked differences in the Pcdh-α protein levels on the cell surface are found between the wild-type and Pcdh-γ targeted neurons. This result indicates that Pcdh-α surface delivery, in cultured neuron and in vivo, is mainly independent of Pcdh-γ expression (Bonn et al. 2007).

Several proteins that interact with the cytoplasmic regions of Pcdh-α and Pcdh-γ have been reported (Kohmura et al. 1998; Gayet et al. 2004; Triana-Baltzer & Blank 2006). A complementary DNA fragment of Pcdh-α was firstly isolated as a weak binder from a yeast two-hybrid system using the N-terminus of Fyn kinase (Kohmura et al. 1998). The expression patterns and subcellular fractionation (enrichment in the PSD fraction) are similar between the Pcdh-α and Fyn proteins. The Pcdh-α4 protein (recognized with the monoclonal antibody 6-1B) is co-precipitated with an anti-Fyn antibody (Kohmura et al. 1998). In addition, although several anti-Pcdh-α and anti-Pcdh-γ antibodies can co-precipitate both Pcdh-α and Pcdh-γ proteins from brain extracts (Murata et al. 2004), Fyn protein is not included in these immunoprecipitants (T. Yagi, unpubl. data, 2007). Thus, whether there is an in vivo interaction between Pcdh-α and Fyn proteins is not yet clear. On the other hand, neurofilament M (NFM) and fascin were isolated by yeast two-hybrid screening with the two (A-type and B-type) cytoplasmic constant regions of Pcdh-α, respectively (Triana-Baltzer & Blank 2006), suggesting that links between Pcdh-α protein and the cytoskeleton are mediated by NFM (neurofilament) and fascin (actin filament); however, these protein interactions and their functions in vivo have yet to be determined. It is difficult to obtain conclusive evidence for Pcdh-α-binding proteins from intact-cell-based assays, because heterologously overexpressed Pcdh-α protein is mostly retained in intracellular compartments, such as the endoplasmic reticulum and Golgi (Takei et al. 2001; Blank et al. 2004).

From a yeast two-hybrid binding assay with the cytoplasmic region of Pcdh-γB1 a microtubule destabilizing protein, SCG10, was isolated (Gayet et al. 2004). Both SCG10 and Pcdh-γB1 protein are concentrated in growth cones, where they are co-localized, and they are highly expressed in the dorsal root entry zone of the spinal cord at specific developmental stages (Stein et al. 1988). However, an interaction between these proteins has not been supported by in vivo immunoprecipitation assays using brain extracts. To clarify the molecular function of the clustered Pcdh proteins, the proteins that bind to their cytoplasmic regions need to be further evaluated and analyzed with respect to their in vivo interactions and functions.

Expression and gene regulation of the clustered Pcdh family

Clustered Pcdhs are expressed during neural development, gradually concentrated in the synaptic region, and their levels greatly decrease as the brain matures (Kohmura et al. 1998; Wang et al. 2002b; Phillips et al. 2003; Blank et al. 2004; Morishita et al. 2004b; Frank et al. 2005; Zou et al. 2007). The levels of the clustered Pcdh gene transcripts peak between postnatal days 0 to 10. The decline in Pcdh-α protein expression is triggered by myelination (Morishita et al. 2004a) (Fig. 4). In the optic nerve, the expression of Pcdh-α proteins increases during nerve development and then dramatically decreases at P10, when the myelin sheath is compacted. In the Shiverer mutant mouse, which carries a deletion of the gene encoding myelin basic protein, the expression of Pcdh-α protein does not decrease rapidly at P10, and is elevated at P19 (Morishita et al. 2004a; Fig. 4). These results may
individual neurons (Esumi et al. 2006). Single-cell PCR analyses of Purkinje cells showed that individual neurons express apparently random sets of $Pcdh$-$\alpha_{1–12}$ mRNA. Although the same $Pcdh$-$\alpha_{1–12}$ isoform is expressed from both chromosomes, this dual expression is rarely seen, suggesting that the choice of promoters is stochastic within each chromosome and independent of the choice in the other chromosome (Esumi et al. 2005; Fig. 3). The total allelic gene regulation in the $Pcdh$-$\alpha$ and $\gamma$ clusters, including the C-type variable exons (C1 to C5) and the $Pcdh$-$\gamma A$ and $\gamma B$ variable exons, was examined in single Purkinje cells. The results showed that all the C-type isoforms are biallelically expressed in almost all Purkinje cells. On the other hands, the $Pcdh$-$\alpha$, $\gamma A$, and $\gamma B$ isoforms are differentially regulated in each cell by both monoallelic and combinatorial gene regulation mechanisms (Kaneko et al. 2006; Fig. 3). In the $Pcdh$-$\alpha$ and $\gamma$ clusters, different types of allelic gene regulation (monoallelic and biallelic) occur, and these genes are spliced to the same constant exons. In particular, elucidating the process by which the stochastic expression of $Pcdh$-$\alpha$, $\gamma A$, and $\gamma B$ is regulated in individual neurons will be valuable for understanding the molecular mechanisms involved in generating neuronal individuality and diversity. There is a possible hypothesis that their differential expression in individual neurons might provide a unique identity to distinguish between self and non-self neurons. In addition, similar to clustered $Pcdh$ genes, it is reported that more than 1000 genes in the human genome can potentially be monoallelically expressed (Gimelbrant et al. 2007). These suggest that gene regulation by random allele inactivation can generate diversity in gene expression that affects cell fate and physiology (Ohlsson 2007). In addition, different states of DNA methylation are observed in variable $Pcdh$-$\alpha$ promoter regions (Tasic et al. 2002; Kawaguchi et al. 2008).

Long-range cis-regulatory DNA elements in the $Pcdh$-$\alpha$ gene cluster were identified (Ribich et al. 2006). Two genomic DNA fragments, HS5-1 and HS7, possess enhancer activity in reporter assays (see Fig. 1). Interestingly, both HS5-1 and HS7 are conserved among vertebrates, and include CSE sequences, which are highly conserved among the promoters in the clustered $Pcdh$ genes. Reporter assays using transgenic mice showed that the HS5-1 element functions in the brain, and the HS7 element functions in the peripheral nervous system. Furthermore, disruption of the HSS-1 region from the $Pcdh$-$\alpha$ cluster genome in embryonic stem cells revealed that HS5-1 is required for high-level expression from the $Pcdh$-$\alpha_{1–12}$ and $-\alpha C1$ promoters, but not from the

![Fig. 4. Localization of $Pcdh$-$\alpha$ protein to axons during brain development and regulation of $Pcdh$-$\alpha$ protein level by axonal development and myelin maturation. (A) Inverse pattern of $Pcdh$-$\alpha$ and myelin basic protein (MAG) expression in developing axons of the internal capsule. The level of $Pcdh$-$\alpha$ protein is decreased during myelination. (B) Elevated expression of $Pcdh$-$\alpha$ protein in the optic nerve of Shiverer mice, which are hypomyelinated mutants. E, embryonic day; P, postnatal day. Adapted from Morishita et al. (2004a).](image)
Pcdh-αC2 promoter. That the HS5–1 element is involved in regulating the majority of the clustered members is consistent with the idea that monoallelic expression of the Pcdh-α variable exon is a consequence of competition among the individual variable exon promoters for the regulatory elements. However, the long-range cis-regulatory DNA elements have not yet been completely identified in the Pcdh-β and Pcdh-γ clusters, or even in the Pcdh-α cluster. To clarify the molecular mechanisms responsible for the unusual regulation of the clustered Pcdh genes, the cis-regulatory DNA elements in the genome of the Pcdh gene cluster need to be further identified and evaluated.

In addition to the long-range cis-regulatory elements, recent human genome-wide analyses of the binding sites of the insulator protein CCCTC-binding factor (CTCF) indicate that CTCF-binding sites punctuate the alternative promoters in the Pcdh-α and Pcdh-γ cluster locus (Kim et al. 2007; Xie et al. 2007). This finding raises the possibility that insulator elements are involved in the selection of promoters in individual neurons. To better understand the molecular mechanisms involved in generating the diversity of individual neurons, further studies aimed at clarifying the mechanisms of regulation in the Pcdh gene cluster will be valuable.

In vivo function of the clustered Pcdh family

To examine the in vivo function of the clustered Pcdh family, genetic studies in mice have been performed. Deleting the cluster of Pcdh-γ genes results in a partial apoptotic loss of spinal interneurons, and a reduction in spinal synaptic density (Wang et al. 2002b). To investigate the roles of Pcdh-γ specific to synaptogenesis, apoptosis can be minimized by removing BAX, or by using a hypomorphic allele of Pcdh-γ (Weiner et al. 2005). In these mutant mice, the spinal cord still shows decreased synaptic density, and the activity of the formed synapses is reduced, providing the first evidence for a role of this clustered Pcdh in synaptic development. However, the localized effect of the Pcdh-γ deletion on interneurons of the spinal cord also indicates that the expression of different members of the clustered Pcdh family might specify both the survival and the synaptic formation of different neuronal populations. Recently we demonstrate that Pcdh-α mutants show abnormal sorting of olfactory sensory neurons into glomeruli (Hasegawa et al. in press). Further analyses of Pcdh-α and Pcdh-β family mutant mice, as well as mice with reduced numbers of clustered Pcdhs, are current research directions.

Conclusion

The clustered Pcdh family is a structurally and functionally related subgroup of the cadherin superfamily, whose members share a unique protein structure and unusual gene regulation mechanisms. Keen interest in the clustered Pcdh family arises from its extensive diversity in the vertebrate brain. In invertebrates such as Drosophila, another cell adhesion molecule, DSCAM, shows extraordinary diversity (Schmucker et al. 2000), which provides each neuron with a unique identity that enables it to distinguish between self and non-self cells (Zipursky et al. 2006). Recent findings on the protein structure and properties, gene regulation, and in vivo functions of the clustered Pcdh family indicate that these diverse proteins play significant roles in the vertebrate brain. Continued investigation of the clustered Pcdh family by various approaches will provide important information about the molecular mechanisms involved in generating the highly complex systems of the vertebrate brain.

Acknowledgments

I am grateful to K Inoue and S Yokota, and Drs S Hamada, K Senzaki, R Kaneko, T Hirayama, and H Morishita for their contribution in preparing the manuscript.
Conflict of Interest

No conflict of interest has been declared by Y. Yagi.

References


