CADHERINS IN BRAIN MORPHOGENESIS AND WIRING
Shinji Hirano and Masatoshi Takeichi

Department of Neurobiology and Anatomy, Kochi Medical School, Okoh, Nankoku-City; and RIKEN Center for Developmental Biology, Minatojima-Minamimachi, Chuo-ku, Kobe, Japan

Hirano S, Takeichi M. Cadherins in Brain Morphogenesis and Wiring. Physiol Rev 92: 597–634, 2012; doi:10.1152/physrev.00014.2011.—Cadherins are Ca\(^{2+}\)-dependent cell-cell adhesion molecules that play critical roles in animal morphogenesis. Various cadherin-related molecules have also been identified, which show diverse functions, not only for the regulation of cell adhesion but also for that of cell proliferation and planar cell polarity. During the past decade, understanding of the roles of these molecules in the nervous system has significantly progressed. They are important not only for the development of the nervous system but also for its functions and, in turn, for neural disorders. In this review, we discuss the roles of cadherins and related molecules in neural development and function in the vertebrate brain.

I. INTRODUCTION

Cell-cell contact and adhesion are a crucial process in the development of multicellular organisms. Fertilized eggs give rise to numerous cells that form tissues and organs, and these cells must keep in physical contact with others for their structural and functional communications. The nervous system is one of the most complex and sophisticated parts of the animal body, requiring various forms of cell-cell contacts for its development and functions.

Complex neural networks in the nervous system were first described in the late 19th century. Santiago Ramon y Cajal described precise structures and organization of the nervous system by using Golgi staining (254). Cajal and others claimed the “neuron theory,” in which nerve cells are connected not by protoplasmic bridges but by close contacts (150). Harrison speculated that cell surface events might be involved in neural connections (116). To explain the mechanisms of how such complex neural wiring develops has been one of the major issues in neuroscience for over a century.

The formation of neural networks is achieved by a series of developmental processes, including cell fate determination, proliferation, migration, differentiation, axon elongation, pathfinding, target recognition, synaptogenesis, synapse elimination, synaptic plasticity, and so on. Many of these steps require cell-cell interactions, and cell-cell contacts provide a platform for these cell-cell interacting processes. The cell-adhesion molecules themselves often play active roles in cell-cell interactions, such as in cell recognition and signal transduction via their cytoplasmic domains. There are several major families of cell-adhesion molecules, including the immunoglobulin superfamily and cadherin superfamily.

Cadherins are a group of transmembrane proteins that were originally identified as the cell-surface molecules responsible for Ca\(^{2+}\)-dependent cell-cell adhesion (338, 339, 397). Subsequently, various molecules sharing amino acid sequences with the cadherins were identified, and this group of proteins is defined as the cadherin superfamily. The roles of these molecules in the development of the nervous system as well as in mature neurons have extensively been studied. In this review article we overview the progress of these studies, focusing on the vertebrate nervous system.

II. DEFINITION AND CLASSIFICATION OF THE CADHERIN SUPERFAMILY

Cadherins are defined as transmembrane proteins whose extracellular domain has a repeated primary sequence termed the “cadherin motif” or “cadherin repeat” (338), which has more recently been referred to as the “cadherin EC domain.” These molecules, which were initially identified as cadherins, have five cadherin motifs/EC domains, and these cadherins are now called the “classical cadherins.” The majority of the other members of the cadherin superfamily have even more EC domains (FIGURE 1). Cadherins require Ca\(^{2+}\) for their functions, and the cadherin motif contains conserved Ca\(^{2+}\)-binding sequences such as AXDXD, LDRE, and DXNDN. Cadherin molecules thus defined are detected throughout multicellular animal species and even in unicellular choanoflagellates (243). Whatever the origin of the cadherin superfamily is, cadherin molecules have successfully evolved in the animal kingdom. In humans, there are more than 110 cadherin superfamily members.
Cadherins are divided into distinct subgroups. Hulpiau and van Roy (2009) classified cadherins into 6 families and 19 subfamilies based on numerous sequence data from various species (138). The classification of cadherins, however, has not yet been completed, and the current classification will require further revisions using different criteria. For simplicity, in this article, we tentatively divide the cadherin superfamily into 12 families, based on their structure and function (TABLE 1), and show in FIGURE 1 a schematic diagram of the representative molecules including classical cadherins, desmosomal cadherins, T-cadherin, 7D-cadherins, protocadherins, CDH15 & 23, Fat & Dachsous, Flamingo/Celsr, calsyntenins, and Ret. Among these molecules, at least the classical cadherins and desmosomal cadherins function as cell-cell adhesion molecules, whereas many others have more diverse functions, such as signaling functions. It is assumed that even these functions are elicited via their homophilic or heterophilic interactions occurring at cell-cell interfaces. Many of these molecules are expressed in the nervous system, where they contribute to neural development and function.

III. CLASSICAL CADHERINS

A. Molecular Properties and Function of Classical Cadherins

Here, we summarize the basic properties of the classical cadherins. It should be noted that many studies on classical cadherins have been conducted for limited subtypes of cadherin such as E-cadherin, and there may be some variations in their properties among the different subtypes.
1. Structure of classical cadherins

Classical cadherins are a group of type I transmembrane proteins with five cadherin motifs or EC domains (EC1-EC5) in their extracellular region and a unique cytoplasmic domain (337, 338) (FIGURE 2A). The cytoplasmic domain is highly conserved among the members of the classical cadherin family, which comprises 18 members in humans, and it interacts with /catenin and p120-catenin, as described below. These classical cadherins can be further divided into two subfamilies named type I (e.g., E-, N-, R-, and P-cadherin) and type II (e.g., cadherin 5, 6, 7, and 8), which show some sequence differences especially in their cytoplasmic domain (329). There seem to be some functional differences between these two subfamilies, although these differences have not fully been established. For example, it was reported that E-cadherin (type I)-mediated adhesion is much more rapid and stronger than that of cadherin 7 (type II) (57). Similarly, cells transfected with cadherin 7 are more motile than N-cadherin (type I)-expressing cells (78).

2. Extracellular domain

Each of the EC domains bears seven β-strands, each forming two β-sheets, and its folding topology is similar to that of the immunoglobulin domain (247, 306). Ca2+ binds to the boundary between EC domains, resulting in the formation of a rodlike structure comprising the entire extracellular domain (228). The extracellular domain thus stabilized via its binding to Ca2+ undergoes a homophilic interaction, which is the key reaction for cadherins to function as adhesion molecules (FIGURE 2B). The details of this interaction, however, still remain controversial. It is thought that a cadherin monomer interacts with another cadherin monomer on the same plasma membrane, forming cis-dimers. In experimental conditions, this transition occurs at ~500 μM.
Ca^{2+}(257), and the formation of such cis-dimers can actually be observed when cadherin-mediated adhesion takes place (42, 343). Even a chimeric cis-heterodimer of N-cadherin and R-cadherin can be formed in L cells, and possibly also in synapses overexpressing these molecules (304), although its presence in native synapses remains unknown. On the other hand, a recent study showed that cis-dimerization is not mandatory for the trans-interactions between classical cadherins (400). Whatever the case is, a pair of monomers or cis-dimers derived from the apposed cells should interact in trans configuration for adhesion.

For the trans-interaction, it is proposed that the extracellular domains first form a X-shaped trans-dimer near EC1-EC2 (115) and then the tryptophan 2 (Trp2) residue in the EC1 of one molecule adapts to the hydrophobic pocket of the other molecule (115, 247, 251, 257, 305). This interaction occurs reciprocally between the two interacting molecules, and therefore is called “strand swap” or “strand exchange.” Although the trans-interaction at EC1s seems to be a major occurrence (400), other EC domains may also contribute to the trans-interactions between the cadherins (52, 317, 360). The binding mechanisms between type II cadherins are similar to those of type I cadherins, but two tryptophan residues (Trp2 and Trp4) are inserted into the hydrophobic pocket at the trans-interactions of type II cadherin EC1 domains (255).

Lateral clustering of cadherin molecules on the plasma membrane is expected to strengthen cell adhesion and leads to the development of junctional complexes (38, 137, 142, 392, 393). A study on desmosomal cadherins by electron tomography suggested alternate cis- and trans-configurations, which could make these molecules form clusters in a quasi-periodical arrangement (10). However, this desmosomal model appears not to be identical to the early observations on classical cadherins by electron microscopy, in which only sparsely positioned rodlke bridges rather than densely packed molecules are seen in the extracellular space at the adherens junction (FIGURE 2C) (131). Apparent differences in the images of adherens junctions and desmosomes suggest that the model based on the desmosome needs to be modified to explain the structure of the adherens junction.

3. Cytoplasmic domain and cadherin-associated molecules

The cytoplasmic domain of classical cadherins is important for them to produce strong cell-cell adhesion (FIGURE 3). Various molecules interacting with the cytoplasmic domain have been identified, which include “catenins” as major ones (see reviews in Refs. 176, 209, 233, 263) (TABLE 2). There are three groups of catenins: α, β, and p120. The β-catenin and p120-catenin groups directly bind the cad-
herin cytoplasmic domain, and α-catenins do so via their binding to β-catenin.

α-Catenins are a small molecular family consisting of three members (αE, αN, and αT) in the vertebrates, and these molecules have a low sequence similarity to vinculin (227, 263). These molecules contain the β-catenin-binding site near their NH2 terminus, vinculin/α-actinin-binding sites in their central region, and a ZO-1-binding site near their COOH terminus. αE-catenin (originally α-catenin) is widely expressed in various tissues, whereas αN- and αT-catenin are expressed in limited tissues; for example, αN-catenin is mainly expressed in neural tissues (127). Because αE-catenin interacts with F-actin, this protein has been considered to be a linker between cadherin and the actin cytoskeleton. However, recent findings suggest that the αE-catenin monomer bound to β-catenin does not interact with F-actin and that only α-catenin dimers, isolated from the cadherin-β-catenin complex, bind actin bundles (77). In fact, αE-catenin can regulate actin dynamics independently of cadherin-mediated cell-cell adhesion (31). In this model, actin bundles are not directly linked to the cadherin-α-catenin complexes. On the other hand, it has been proposed that some actin-binding molecules, such as vinculin and EPLIN, mediate the interactions between αE-catenin and F-actin (2, 263). Other α-catenin-binding proteins, listed in Table 2, could also play a similar role.

The β-catenin group comprises β- and γ-catenin (plakoglobin). β-Catenin binds to the COOH-terminal region of classical cadherins. It contains 12 Armadillo repeats, and repeats 2–9 are responsible for the binding to cadherins (209). The primary role of β-catenin at the cell-cell junction is to link cadherin with α-catenin. γ-Catenin/plakoglobin, a molecule similar to β-catenin, also binds to classical cadherins, and β- and γ-catenins appear functionally redundant. However, the real functional differences between these two catenins are not perfectly understood (209). It should be noted that γ-catenin also binds to desmosomal cadherins, whereas β-catenin has no such ability. In addition, β-catenin is well known as a transcriptional regulator downstream of Wnt signaling (122, 192, 210), whereas γ-catenin has no such functions (311).

FIGURE 3. Regulation of classical cadherin-mediated adhesion by cytoplasmic components. A: schematic drawings to explain how cadherins mediate strong adhesion via their linkage to the actin cytoskeleton. B: photographs of lung carcinoma PC9 cells with or without α-catenin. These cells, which do not express α-catenin, show only weak adhesion. However, when α-catenin is introduced into these cells, they show strong adhesion, restoring the epithelial architecture.
The p120-catenin group contains p120-catenin, δ-catenin, ARVCF, p0071, and plakophilins (48, 119). These molecules are characterized by their having the Armadillo repeats, as the β-catenin group does. p120-catenin binds to the juxtamembrane region of the cadherin cytoplasmic domain (176, 209). Multiple roles for p120-catenin have been reported. The most established function of this catenin is to stabilize classical cadherins at the plasma membranes (66, 172, 393); in its absence, cadherins are internalized through endocytosis. p120-catenin is also known to affect Rho family GTPase activities (384). A recent study showed that p120-catenin plays a role in the linkage between cadherins and microtubules via PLEKHA7 and Nezha (213). How these multiple functions of p120-catenin are related to one another is not known, and this point should be clarified in the future. Other members of the p120-catenin group are also known to bind classical cadherins, although the association of plakophilins with the classic cadherins seems to occur only in certain tumors (282); plakophilins generally localize at desmosomes and participate in linking desmosomal cadherins to intermediate filaments (68, 209).

In addition to catenins, various other molecules are known to interact with classical cadherins (Table 2). Such diverse classes of cadherin-binding molecules are thought to mediate the “cross-talk” between cadherins and other cellular systems (87, 324). For example, the cadherin-catenin complexes interact with various receptor-type tyrosine kinases such as FGF receptors and EGF receptors and regulate their activities. The cadherin system also interacts with molecules involved in RhoGTPase (381) and Wnt signaling (122), as well as with those functioning in NF-κB, Ras, and Hedgehog signaling (324). These interactions are probably related to the diverse roles of cell-cell adhesion as a regulator of cellular behavior (87, 122, 129, 324, 381).

### Table 2. Binding partners of cadherin and catenins

<table>
<thead>
<tr>
<th>Cadherin/Catenin</th>
<th>Binding Partner</th>
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<tbody>
<tr>
<td>α-Catenin</td>
<td>β-Catenin or plakoglobin</td>
</tr>
<tr>
<td>β-Catenin</td>
<td></td>
</tr>
<tr>
<td>Plakoglobin</td>
<td></td>
</tr>
<tr>
<td>p120Ctn</td>
<td></td>
</tr>
<tr>
<td>δ-Catenin</td>
<td></td>
</tr>
<tr>
<td>FGF-R</td>
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<tr>
<td>Ga12,13</td>
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<tr>
<td>Presenilin</td>
<td></td>
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<tr>
<td>PTP-1B</td>
<td></td>
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<tr>
<td>PTP-µκ</td>
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<tr>
<td>LAR-PTP</td>
<td></td>
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<tr>
<td>Shc</td>
<td></td>
</tr>
<tr>
<td>Hakai</td>
<td></td>
</tr>
<tr>
<td>GlcNacPTase</td>
<td></td>
</tr>
<tr>
<td>Vezatin</td>
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</tr>
<tr>
<td>ADAM10</td>
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</tr>
<tr>
<td>MT5-MMP</td>
<td></td>
</tr>
<tr>
<td>NMDAR</td>
<td></td>
</tr>
<tr>
<td>Nectin ? (check)</td>
<td></td>
</tr>
<tr>
<td>AKAP97/150</td>
<td></td>
</tr>
<tr>
<td>Liprin-α &amp; GRIP</td>
<td></td>
</tr>
<tr>
<td>AMPAR (GluR2)</td>
<td>p120-catenin</td>
</tr>
<tr>
<td>Arcadin (protocadherin B)</td>
<td>DEP-1</td>
</tr>
<tr>
<td>PAPC (paraxial protocadherin)</td>
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</tr>
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<tr>
<td>PDZ-GEF</td>
<td></td>
</tr>
<tr>
<td>α-Catenin</td>
<td>Kinesin</td>
</tr>
<tr>
<td>β-Catenin</td>
<td></td>
</tr>
<tr>
<td>γ-Catenin(plakoglobin)</td>
<td></td>
</tr>
<tr>
<td>α-Actinin</td>
<td></td>
</tr>
<tr>
<td>EPLIN</td>
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<tr>
<td>Actin</td>
<td></td>
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<tr>
<td>Afadin</td>
<td></td>
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<tr>
<td>Formin-1</td>
<td></td>
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<tr>
<td>Spectrin</td>
<td></td>
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<tr>
<td>Vezatin</td>
<td></td>
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<tr>
<td>Vinculin</td>
<td></td>
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<tr>
<td>ZO-1</td>
<td></td>
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<td>Zyxin</td>
<td></td>
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<tr>
<td>MyosinVlla</td>
<td></td>
</tr>
<tr>
<td>ALCAM, SC-1</td>
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<tr>
<td>MINT (?)</td>
<td></td>
</tr>
<tr>
<td>Merin</td>
<td></td>
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This is not a complete list. See also Hirano et al. (129), Takeichi and Abe (340), and Arikkath and Reichardt (20).

### 4. Adherens junction

Cell junctional structures organized by classical cadherin-catenin complexes together with the actin cytoskeleton are called adherens junctions (AJs). In simple epithelia, the AJs are designated as the zonula adherens, belt desmosome, or intermediate junction; in cardiac myocytes, as the fascia adherens; and in other cell types, as spot adhesions or puncta adherentia (106). This puncta adherentia is a special form of AJ localized at synapses and will be described in later sections. E-cadherin is the major component of the zonula adherens in nonneural epithelia, and N-cadherin organizes similar AJs in neural epithelia, as well as spot-type AJs in fibroblasts and neural cells. Other classical cadherins are also involved in AJ formation.

The AJs are a dynamic structure that is regulated by the actin cytoskeleton (106, 114, 235, 336, 361). The formation of AJs in epithelial and fibroblastic cell lines has been studied extensively (see review by Meng and Takeichi, Ref. 214). In the initial contacts between cells, nectins, which are
immunoglobulin superfamily members related to Necls/ SynCAMs, interact in trans at cell-cell interfaces (336) (FIGURE 4). Cadherins are recruited to these nectin-mediated contact sites via the interaction between nectin-bound afadin and cadherin-bound α-catenin or p120-catenin (296, 336). A recent study suggests that N-cadherin is accumulated at the apical region of the neural tube via its interaction with nectin-2 at their extracellular domains (219). Thus it seems that the formation of the cadherin-based AJs is assisted by other receptor-type molecules, such as nectins.

5. Roles of classical cadherins in cell sorting

In principle, cadherins are homophilic adhesion molecules, and cadherin-mediated adhesions show subtype specificities (338). Cells expressing a given cadherin adhere preferentially to those expressing the same cadherin subtype. For example, E-cadherin-expressing cells adhere to other E-cadherin-expressing cells, and P-cadherin-expressing cells adhere to those expressing P-cadherin; and most importantly, the cells with E- and P-cadherin form independent aggregates when mixed in culture (237) (FIGURE 5A). EC1 is primarily responsible for the specificities of these molecules (238, 303). Heterotypic adhesions, however, do occur in other combinations of cells expressing different classical cadherin subtypes. For example, this is seen between cells with N- and R-cadherins and between those with cadherins 8 and 11 (146, 312). These cells do not distinctly segregate during aggregation. Weak heterotypic adhesions are also observed between N-cadherin- and E-cadherin-expressing cells (160); in this combination, cells expressing these cadherins form homotypic aggregates, but these homotypic aggregates attach to one another heterotypically.

Some reports have shown that cells with different cadherin subtypes do not segregate, unless their expression levels are quantitatively different (80, 93). The discrepancy between these observations remains to be explained, but experimental conditions likely affect the results of these assays; for example, when non-cadherin adhesion molecules are active in the cells used for an assay, these molecules should interfere with the cadherin-specific processes of cell sorting. In addition, it is often observed that different cell types are in contact with each other in tissues and cultures, even when each cell type expresses a cell type-specific cadherin (for example, Refs. 128, 376). It should be noted that any given cell generally expresses multiple cadherin subtypes. If such cadherins are expressed in both cell types undergoing heterotypic adhesion, these molecules might be responsible for their heterotypic adhesion.

The molecular basis for the cadherin specificities is still unclear (307). N-cadherins show homophilic interactions with higher affinity than E-cadherins. Interestingly, the N- to E-cadherin heterophilic binding affinity is intermediate in strength between the two homophilic affinities (160). Then, why do cells expressing E- and N-cadherin segregate in their mixture? In addition, no cadherin subtype specificities were found when the adhesion of cells to purified cadherin-coated substrates was measured (236), suggesting that the cadherin specificities might be detectable only when the interactions between live cells are observed. Reconciling the molecular events and cellular behavior is an important future subject to address for a comprehensive understanding of cadherin subtype-dependent cell sorting mechanisms.

Quantitative differences in the amount of cadherins expressed between cells also can contribute to cell sorting (80,
93, 323). Cells with more cadherins can segregate from cells with less cadherins in an inside-out fashion, as predicted by the differential adhesiveness hypothesis (322). The combination of qualitative and quantitative differences likely confers the cell type-specific adhesion affinities on individual cells (FIGURE 5B).

B. Expression in the Embryonic and Mature Brains

Expression of classical cadherins is dynamic during development (338). In neural tissues, various cadherins are widely expressed (129, 275–277, 279). These cadherins begin to be expressed upon neurulation: the embryonic ectoderm first expresses E-cadherin, but this E-cadherin is replaced by N-cadherin and other classical cadherins soon after neural induction (118) (FIGURE 6A). In the neural tube, N-cadherin is broadly expressed in neuroepithelial cells, although this protein is most highly concentrated at the apical side of these cells, where the AJs develop. Other classical cadherins are also expressed in various local regions of the neural tube. As cell differentiation proceeds in the neural tube, the regional expression of classical cadherins coincides with the locations of embryonic neurons and their boundaries, although the expression patterns of different cadherins often overlap with each other (FIGURE 6B AND C). During the maturation of brains, expression of each cadherin subtype becomes further restricted to smaller brain subdivisions such as brain nuclei and lamina of the cortex, as neuronal precursor cells migrate to and differentiate at these sites (276). A particular subtype is expressed in a specific subset of brain nuclei and layers, and its expression occurs along specific neural networks (FIGURE 6D). This correlation between cadherin expression and functional neural circuits suggested that classical cadherins might contribute to the selective association between axons and their targets through their homophilic bindings (275, 277, 331) (see also below). It should be noted that each nucleus or layer in the brain contains heterogeneous neurons, and the expression of a given cadherin in each region generally occurs only in a subset of these neurons (331). These suggest that classical cadherins are utilized for the recognition of individual neurons, rather than tissue regions. In the adult brain, the expression of cadherins is much reduced in general, but cadherin proteins often remain localized as puncta that correspond to synapses or perisynaptic areas. Overall, the past observations suggest that cadherins are involved in various aspects of neural development and functions (see below for functional analysis).

C. Roles in Brain Development

Cadherin-mediated adhesion is involved in various processes of neural development (FIGURE 7A, TABLE 3). Neural development is achieved by a series of successive processes including neurulation, neuronal differentiation and migration, axon outgrowth, pathfinding, target recognition, and synapse formation. Here, we discuss the roles of classical cadherins in each step of neural development, except for synapse formation, which will be discussed in a separate section.
FIGURE 6. Expression of classical cadherins in the neural tube and embryonic brain. A: upon neurulation, expression of E-cadherin at the overlying ectoderm ceases, replaced by that of N-cadherin. [From Alberts et al. (13), copyright 2008 from Molecular Biology of the Cell by permission of Garland Science/Taylor & Francis Books, LLC.] B: expression of R-cadherin is detected in distinct regions of the neuroepithelium, including radial glia, migrating neurons, brain nuclei, and fiber tracts, in the chicken embryonic brain. [From Redies (276), with permission of Elsevier.] C: expression of various cadherins in prosomeres. Note that each cadherin is expressed in particular prosomeres as well as along their boundaries. [From Redies and Takeichi (277), with permission of Elsevier.] D: expression of cadherins in the postnatal mouse brain. Cadherins 6, 8, and 11 are represented in different colors. Four different levels of the forebrain are shown. At the cellular level, each region is a mosaic of neurons expressing and not expressing a particular cadherin. See the original paper for abbreviations (331).
1. Neural tube and neuroepithelial layer formation

As described above, during neural tube formation, E-cadherin is replaced by N-cadherin and other cadherins (118) (FIGURE 7A). E-cadherin remains in the overlying ectoderm and future epidermis to maintain their architecture together with other classical cadherins such as P-cadherin (125). In N-cadherin knockout mice, neurulation proceeds, but the neural tube is distorted, although further analysis of the phenotypes has not been conducted, because embryos die due to heart defects (270). In zebrafish N-cadherin mutants, cell rearrangement, such as convergent cell movements, necessary for neural tube formation, is impaired (135, 184).

The neural tube gives rise to neuroepithelial layers comprising tall epithelium-like cells. The AJs are organized at the apical side of these cells. These AJs become enriched in various steps of neural development. These include formation of tissue architecture, neural compartmentalization, neuroblast migration, axon pathfinding and fasciculation, target recognition, and synapse formation. E-cadherin represents a specific set of classical cadherins, providing an “adhesion code.” Through the homophilic interaction of each cadherin subtype, neurons selectively adhere to a restricted group of other neurons. Note selective adhesion is often observed even with partial matching of cadherin subtypes depending on the relative amount of cadherins. C. classical cadherins in synapse formation. Cadherins mediate initial contacts between dendritic filopodia and axons. During synapse maturation, these cadherins become accumulated at the outer rim region of the active (neurotransmitter-releasing) zone.

FIGURE 7. Classical cadherins in neural development. A. classical cadherins (marked in red and green) are involved in various steps of neural development. These include formation of tissue architecture, neural compartmentalization, neuroblast migration, axon pathfinding and fasciculation, target recognition, and synapse formation. B. cadherin adhesion code hypothesis. Each neuron expresses a specific set of classical cadherins, providing an “adhesion code.” Through the homophilic interaction of each cadherin subtype, neurons selectively adhere to a restricted group of other neurons. Note selective adhesion is often observed even with partial matching of cadherin subtypes depending on the relative amount of cadherins. C. classical cadherins in synapse formation. Cadherins mediate initial contacts between dendritic filopodia and axons. During synapse maturation, these cadherins become accumulated at the outer rim region of the active (neurotransmitter-releasing) zone.
2. Cell migration

Neuronal precursors migrate to the places where they differentiate. In the cortices, neuroblast migration occurs in a radial direction via guidance by radial glia and/or by translocation of neuroblast somata. Interneurons migrate in a tangential direction over long distances. Cadherins seem to be involved in neuronal migration. A recent study showed that N-cadherin is involved in radial migration of neuroblasts in the cerebral cortex, where the endocytosis and trafficking of N-cadherin via Rab GTPases are critical for the migration (162). Cadherin dysfunction inhibits the tangential migration of precerebellar neurons, as well as the directional chain migration of cerebellar granule neurons (283, 346). In addition, a deficiency in αN-catenin causes abnormal migration of Purkinje cells (29). N-cadherin mutation also leads to mispositioning of neurons in the neural tube of zebrafish (184). Thus the cadherin-catenin system appears to be important for neuron precursors to relocate themselves. On the other hand, in vitro analysis suggests that N-cadherin suppresses the migration of oligodendrocytes on astrocyte monolayers (299).

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Neural Phenotypes</th>
<th>Reference Nos.</th>
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<tbody>
<tr>
<td>N-cadherin</td>
<td>Defects in neural tube formation</td>
<td>Radice et al. (270)</td>
</tr>
<tr>
<td></td>
<td>Defects in cortical organization</td>
<td>Kadowaki et al. (157)</td>
</tr>
<tr>
<td>Cadherin-11</td>
<td>Elevation of LTP</td>
<td>Manabe et al. (198)</td>
</tr>
<tr>
<td>Cadherin-6</td>
<td>Normal overall brain structure</td>
<td>Inoue et al. (145)</td>
</tr>
<tr>
<td>Cadherin-8</td>
<td>Defects in synaptic transmission</td>
<td>Suzuki et al. (330)</td>
</tr>
<tr>
<td>M-cadherin</td>
<td>No gross defects but upregulation of N-cadherin in the cerebellum</td>
<td>Hollnegal et al. (133)</td>
</tr>
<tr>
<td>αN-catenin</td>
<td>Abnormal laminar organization in the cerebellum and hippocampus</td>
<td>Park et al. (252)</td>
</tr>
<tr>
<td></td>
<td>Spine elongation</td>
<td>Tobashi et al. (354)</td>
</tr>
<tr>
<td></td>
<td>Defects in the anterior commissure</td>
<td>Uemura et al. (366)</td>
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<tr>
<td>αE-catenin</td>
<td>Abnormal cell proliferation, decreased apoptosis, and cortical hyperplasia</td>
<td>Lien et al. (188)</td>
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<tr>
<td>β-Catenin</td>
<td>Impaired radial migration of neuroblasts</td>
<td>Machon et al. (193)</td>
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<td></td>
<td>Failure of sensory neurogenesis (via Wnt &amp; cadherin)</td>
<td>Hari et al. (113)</td>
</tr>
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<td></td>
<td>Breakdown of neuroepithelial structure (via cadherin)</td>
<td>Junghans et al. (153)</td>
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<tr>
<td>p120-Catenin</td>
<td>Cell fate changes and ventralization of telencephalon (via Wnt)</td>
<td>Backman et al. (23)</td>
</tr>
<tr>
<td>δ-Catenin</td>
<td>Brain malformation and craniofacial development (via Wnt)</td>
<td>Brault et al. (46)</td>
</tr>
<tr>
<td>γ-Protocadherin</td>
<td>Defects in spine maturation (via cadherin) and density (via Rho)</td>
<td>Elia et al. (83)</td>
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<tr>
<td>CNR (Pcdhα)</td>
<td>Cognitive &amp; synaptic dysfunction</td>
<td>Israely et al. (149)</td>
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<td></td>
<td>Aberrant maintenance of dendrites and dendritic spines</td>
<td>Matter et al. (207)</td>
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<tr>
<td>OL-protocadherin</td>
<td>Defects in survival of neurons &amp; synapse formation</td>
<td>Wang et al. (379)</td>
</tr>
<tr>
<td>Arcadlin (Pcdh8)</td>
<td>Defects in synapse formation</td>
<td>Weiner et al. (382)</td>
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<td></td>
<td>Defects in neuronal survival</td>
<td>Lefebvre et al. (182)</td>
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<tr>
<td>CNR (Pcdhα)</td>
<td>Abnormal axonal pathfinding</td>
<td>Hasegawa et al. (117)</td>
</tr>
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<td></td>
<td>Learning abnormalities</td>
<td>Fukuda et al. (39)</td>
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<tr>
<td>OL-protocadherin</td>
<td>Abnormal projection in late serotonergic neurons</td>
<td>Kato et al. (159)</td>
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<td></td>
<td>Failure of striatal axon growth</td>
<td>Uemura et al. (365)</td>
</tr>
<tr>
<td>Arcadlin (Pcdh8)</td>
<td>No gross phenotypes</td>
<td>Yamamoto et al. (391)</td>
</tr>
<tr>
<td></td>
<td>Increased spine number</td>
<td>Yasuda et al. (394)</td>
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<tr>
<td>Protoadherin 21</td>
<td>Abnormal photoreceptor cells and degeneration</td>
<td>Rattner et al. (273)</td>
</tr>
<tr>
<td>Celsr-1</td>
<td>Neural tube defects and abnormal PCP in inner hair cells</td>
<td>Curtin et al. (64)</td>
</tr>
<tr>
<td>Celsr-3</td>
<td>Defects in neural circuit formation</td>
<td>Tissir et al. (352)</td>
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<td>Zhou et al. (401)</td>
<td>Zhou et al. (402)</td>
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<tr>
<td>Cadherin 23</td>
<td>Hearing loss (Waltzer mutant mice)</td>
<td>Di Palma et al. (72)</td>
</tr>
<tr>
<td>Protoadherin 15</td>
<td>Hearing loss (Ames waltzer mutant mice)</td>
<td>Alagramam et al. (11)</td>
</tr>
<tr>
<td>FAT1</td>
<td>Abnormal forebrain development</td>
<td>Gani et al. (59)</td>
</tr>
<tr>
<td>FAT4</td>
<td>PCP phenotypes in inner ear</td>
<td>Saburi et al. (289)</td>
</tr>
</tbody>
</table>

This is not a complete list.
Dynamic regulation of classical cadherins is also required for the migration of neural crest cells, another type of neural precursors. Neural crest cells originate from the neural fold, and they migrate along stereotypical pathways (344). In chicken embryos, downregulation of N-cadherin, which is strongly expressed in the neural tube, appears to be critical for neural crest emigration from the neural tube (62, 229). N-cadherin and also cadherin 11 seem to restrain cranial neural crest cells from migrating out of the neural tube, by stabilizing their cell-cell adhesion as well as repressing canonical Wnt signaling (40, 313). Downregulation of N-cadherin occurs via ADAM10-dependent cleavage of this molecule, and this process is initiated by BMP4 signaling (313). Interestingly, cytoplasmic fragments of N-cadherin produced by ADAM10 cleavage facilitate neural crest migration via a stimulation of canonical Wnt signaling. During migration of neural crest cells, N-cadherin expression is kept suppressed via integrin signaling (217). When their migration ceases, N-cadherin expression is restored, and this process is critical for the formation of discrete sympathetic ganglia (158). Another cadherin, cadherin 6B, plays an intriguing role: this type II classical cadherin is locally expressed in the neural crest-forming regions of the neural tube. This expression of cadherin 6B is important for deepithelialization of premigratory neural crest cells through the stimulation of BMP signaling (253).

Thus cadherins are involved in apparently opposite aspects of cell behavior, promotion and suppression of cell migration. Cells would require cadherins for attaching to other cells during migration, whereas cells would be unable to migrate when their adhesion to others are too strong. Through the precise controls of cadherin expression levels, cells could decide whether they migrate or not. However, the real cellular mechanisms should be far more complex, as additional signaling mechanisms also seem to operate.

3. Boundary formation and early compartmentalization

During brain development, the neuroepithelium gives rise to complex functional subdivisions that provide a framework for future neural networks. In the anterior neural tube, subdivisions named neuromeres can be seen, which are called prosomeres in the forebrain and rhombomeres in the hindbrain. Various cadherins are expressed in the central nervous system according to neuromeric organization (275) (FIGURE 6C). Some are expressed along boundaries, whereas others are expressed within prosomeres in a uniform or gradient manner (206). For example, in the forebrain, R-cadherin is expressed in the future cortex, whereas cadherin 6 delineates the lateral ganglionic eminence (LGE) (145, 326). It has been suggested that the interface between R-cadherin and cadherin 6 expressions can function as a boundary for cell mixing (145). When cadherin 6 is ectopically expressed in cortical cells, these cells become sorted into cadherin 6-positive LGE compartments. Similarly, ectopic expression of R-cadherin in prosomere P2 cells causes these cells to become integrated into the R-cadherin-expressing regions such as the cortex and the ventral thalamus (208). These findings suggest that cadherins may regulate prosomeric compartmentalization by conferring region-specific adhesiveness on embryonic brain cells. However, thus far, no report has been published to demonstrate that prosomeric boundaries are perturbed after knockout of a single cadherin subtype. If cadherins function in boundary formation, multiple cadherin subtypes should therefore be redundantly involved.

4. Formation of brain nuclei and ganglia

Cadherins might be involved in the segregation of cell populations representing brain nuclei. In the lateral motor column of the spinal cord, subsets of cadherins are expressed in motor pools. MN-cadherin was shown to regulate the segregation of these motor pools (266). In Xenopus, F-cadherin is expressed in the sulcus limitans, a boundary between the alar and basal plates in the neural tube, and this cadherin partly mediates positioning of neural cells at the sulci limits (88). Conversely, ectopic expression of N-cadherin in neural tubes leads to abnormal formation of cell aggregates (71, 98). In the peripheral nervous system, N-cadherin was shown to mediate the formation of discrete sympathetic ganglia from neural crest cells, cooperatively with Eph/ephrin-mediated repulsive interactions (158). It was also reported that N-cadherin is involved in the aggregation of placode-derived sensory neurons during the formation of cranial sensory ganglia in concert with slit-Robo signaling (308). The role of classical cadherins in clustering of neuronal precursors is very likely, considering their adhesive functions. However, the question of whether these molecules are also involved in the sorting of these cells remains to be answered, as genetic evidence for this problem is thus far lacking.

5. Cell differentiation

The involvement of cadherins in cell differentiation is not well established. As described before, neural induction seems to normally occur in N-cadherin-deficient mice, and at least some neurons and glia differentiate in these mice (157, 270). In addition, neuronal cells expressing ectopic cadherins maintain their original identity even after changing their positions, suggesting that cadherins are not sufficient for altering neural identity (208). On the other hand, N-cadherin mutations perturb not only chain migration but also differentiation of cerebellar granule cells in zebrafish (283). Thus cadherins seem to be involved in cell differentiation in some cases, but the general role of cadherins in neural differentiation is a subject of future studies. A recent study showed that N-cadherin-mediated adhesion between neural precursor cells inhibits their own differentiation by promoting the β-catenin signaling that maintains them as stem cells in the neuroepithelium (399).
6. Neurite elongation and branching

Neurite outgrowth occurs by growth cone migration and elongation of shafts. N-cadherin mediates neurite elongation, as shown by in vitro (35, 204, 356) as well as in vivo (202, 284, 342) studies. Neurite outgrowth activity was also reported for R-cadherin, E-cadherin, cadherin 7, and cadherin 11 (14, 28, 40, 240, 241, 278). A recent study indicated that N-cadherin is involved in activity-induced dendrite growth (341).

The molecular mechanisms underlying cadherin-mediated neurite outgrowth are complex and not fully understood (112). It has been shown that a mechanical coupling between N-cadherin and F-actin flow through catenins in neurites is a major determinant of growth cone motility and neurite extension (27). It is also known that cadherin-dependent neurite growth is promoted by the lateral interaction of N-cadherin with FGF receptors (FGFRs) (76, 112, 293, 373). Because FGFRs are not localized at N-cadherin-mediated cell contact sites, the role of FGFRs in this process is probably independent of the adhesive function of cadherins. In addition, it was reported that N-acetylgalactosaminylphosphotransferase (104) and PTP1 (240), a protein tyrosine phosphatase, are associated with catenins and that these sustain cadherin-mediated neurite outgrowth. The PTP1-dependent signaling mechanisms to control cadherin-mediated neurite outgrowth seem to be different among different cadherin subtypes.

In addition to the outgrowth of neurites, catenins and catenins are involved in neurite morphogenesis. For example, in retinal horizontal cells, N-cadherin defines the dendritic field size through the control of the attachment of their dendritic processes to photoreceptor cells (342). N-cadherin is also important for CA3 dendrite arborization in the hippocampus (30). Moreover, cadherin 7, which is expressed in mature cranial motor neurons in early development, suppresses axon branching, whereas cadherin 6B seems to enhance axon branching in later development (28). In addition, δ-catenin regulates dendritic branching and morphogenesis (20, 164, 165).

7. Selective fasciculation and axon guidance/pathfinding

Growth cones find their paths by sensing environmental guidance cues. It is thought that pioneer neurons find their way via guidepost cells and intermediate targets, and later axons follow the paths preformed by pioneer axons via selective fasciculation. There is accumulating evidence that cadherins are important for axon guidance. It was shown that the expression of R-cadherin is regulated by the early patterning gene Pax6 and that R-cadherin provides a guidance cue for pioneer axons (14). In zebrafish mutants of N-cadherin, axons of retinal ganglion cells show fasciculation defects and incorrect neural projections (202), and some commissural pathfinding defects (151, 184). In addition, N-cadherin is important for the pathfinding of sensory fibers at choice points (178), as well as for that of other axonal projections (184). Similarly, R-cadherin is involved in normal retinotectal projection (22). The role of cadherins in selective fasciculation was also suggested in the tectofugal pathway in vivo (357), which consists of various tracts that express different sets of cadherins including N-cadherin, R-cadherin, cadherin 6B, and cadherin 7. Ectopic expression of cadherins in particular axons shows that transgenic axons are selectively fasciculated with the tracts expressing the matching types of cadherins. Moreover, in αN-catenin-deficient mice, axons of the anterior commissure are less fasciculated in early phases of development, and then misrouted toward the septum (364).

8. Target recognition

Specific neural connectivity is one of the most important issues under study in neuroscience. Various molecules are speculated to be involved in the target recognition between proper neurons (294). These molecules include immunoglobulin superfamily molecules (e.g., sidekick, Dscam), ephrin/Eph, semaphorin/neuropilin/plexin, netrin/DCC, Toll/Capricious, and Wnt/Frizzled. As described before, the cell-binding specificity is a critical feature of cadherin-mediated adhesions. Accordingly, cadherins have been considered to be candidates for target-recognition molecules. Because a single neuron expresses multiple cadherin subtypes, the combination of cadherins can theoretically provide a complex adhesive specificity, defined as the “adhesion code,” which could be involved in target recognition (123, 173, 277, 339) (FIGURE 7B).

However, in the vertebrate system, evidence for the role of cadherins in target recognition is still limited. N-cadherin is involved in lamina-specific connections in the chick optic tectum (144): in co-culture systems, retinal axons terminate in retinorecipient laminae (SGFS laminae A–J), forming rudimentary arbors. In the presence of N-cadherin antibody, this lamina distribution of retinal axon terminals is altered, and secondary branching of axon is reduced. A similar role of N-cadherin in target specificity can be observed in the mouse thalamocortical axons (264): in the presence of blocking antibodies against N-cadherin, thalamocortical axons continue to grow rather than stopping at layer IV. Thus N-cadherin could function as a layer IV stop signal for thalamocortical axons.

The role of N-cadherin in target recognition is more clearly shown in the Drosophila visual system (294, 337). In this system, DN-cadherin is involved in the target recognition by R7 photoreceptor cells in the lamina and the medulla (181). Genetic analysis suggests that N-cadherin functions as a permissive adhesion molecule rather than an instructive one (351). Similar observations on the role of DN-cadherin in target recognition have been reported for the olfactory...
projection (404). Recent studies show that, even if N-cadherin is a permissive adhesive component, this molecule can function in specific target-recognition processes by spatiotemporal regulation of its function or axonal competence (234, 259).

D. Roles in Synaptogenesis and Synapse Plasticity

Neuronal activity is transmitted from axons of a neuron to dendrites of other neurons via synapses, which structures are critical sites for the regulation of neural function. Chemical synapses in the brain are asymmetric structures consisting of the presynaptic and postsynaptic compartments formed on a pair of axons and dendrites, respectively (FIGURE 7A). To develop the presynaptic part, axons form a swelling on themselves, named the “bouton,” which contains synaptic vesicles. The postsynaptic part contains receptors for neurotransmitters, such as glutamate receptors and GABA receptors, which are anchored to the postsynaptic density (PSD). These basic structures are conserved among the synapses on various types of neurons, but their detailed morphologies vary depending on their functions. In the dendrites of excitatory neurons, a fine protrusion called the “spine” forms to organize the postsynaptic compartment. Spines are highly dynamic structures, and their dynamics are thought to play a role in neural plasticity such as occurs in long-term potentiation (LTP) and long-term depression (LTD). This dynamic is considered to be a cellular basis of learning and memory (see, for example, Ref. 67). In these processes, cell-adhesion molecules, including classical cadherins, play roles (65).

1. Synapse formation

In mature synapses, classical cadherins and associated catenins are localized at the outer rims of the active (neurotransmitter-releasing) zone (91, 362), although these are detectable throughout the cleft of synapses at their immature stages (see below). In synapses, there is a special form of adherens junction named the puncta adherentia that mediates synaptic adhesion, and this is the site where the trans interaction of cadherins occurs between pre- and postsynaptic membranes. Because of these localizations, cadherins are considered to be involved in various processes that are related to synaptic structures and functions (20, 47, 332, 333, 337).

Cadherins are involved in the formation of synapses. Synapses form via various steps including target recognition, synaptic differentiation, and synapse remodeling (94). Dynamic filopodia protruding from dendrites make their first contacts with axons of excitatory neurons, and this process presumably includes recognition between axons and dendrites. These early contacts are then stabilized via the morphological transformation of the filopodia into spines (FIGURE 7C). During this process, cytoskeletal elements are reorganized, and various synapse-specific molecules become accumulated. This differentiation is achieved depending on “synaptic organizers” such as neuroligin and ephrin B (94). In the early stages of synapse formation, N-cadherin is localized at the contact sites between filopodia and axons, and then later becomes restricted to synaptic sites (354). Since cadherin-cadherin interactions are subtype specific, the classical cadherins may serve not only as permissive adhesion molecules but also as recognition molecules during these processes.

Usually, axons form synapses only with dendrites and not with other axons, suggesting that there is a recognition mechanism(s) for discrimination between these two classes of neurites. One possible mechanism to explain this recognition has been offered: nectin-1 is localized at presynapses, whereas nectin-3 is localized at postsynapses (215). Since these two nectins prefer heterophilic interactions to homophilic ones, they promote the axon-dendrite interactions but not axon-axon or dendrite-dendrite interactions (355). Cadherins as homophilic adhesion molecules are not responsible for this recognition process, but they are required as permissive adhesion molecules for maintaining the contacts between axon and dendrite initiated by the nectins (355).

N-cadherin seems to be a major synaptic cadherin, but other cadherins also should be involved in synapse formation and maintenance, particularly because β-catenin, a general partner for all classical cadherins, is still localized at synapses in N-cadherin knockout neurons (157). In fact, multiple cadherin subtypes are expressed in single neurons (123, 330), and these cadherins should be involved in synapse formation and functions. For example, cadherin 8 is required for the function of a specific somatosensory circuit (330), and for formation of synapses in the hippocampal mossy fibers (30). Cadherin 11 is required for regulation of LTP (198), and cadherins 11 and 13 (T-cadherin) were shown to regulate formation of both excitatory and inhibitory synapses (250). It has been observed in some systems that, when cadherins are blocked, the synapses are small and that their maturation and function are impaired (see, for example, Refs. 43, 44). Interestingly, a recent study showed that N-cadherin and neuroligin cooperate to regulate synapse formation (9).

One of the roles of classical cadherins in synaptogenesis is to sustain spine morphogenesis. After initial contacts, cadherin is required for the transformation of filopodia to spines in excitatory neurons (1, 244, 245, 354). Inhibition of cadherin function results in abnormal morphogenesis of these spines, such as their filopodium-like elongation and bifurcation at the head (1, 354), and also in a reduction in spine length and even spine loss (225). Cadherins regulate spine morphology via their interaction with αN-catenin and p120-catenin. αN-catenin loss causes instability of spines...
1. p120-catenin deficiency inhibits spine maturation, probably via the reduction in cadherin levels and aberrant Rho-family GTPase signaling (83). Similarly, β-catenin is involved in spine morphogenesis via the regulation of small RhoGTPases (5, 19). Moreover, tumor necrosis factor (TNF)-α/TNF receptor signaling, which is implicated in the pathogenesis of major depression, was shown to be important to maintain N-cadherin expression that is required for normal spine morphogenesis (175). Thus spine morphogenesis by cadherins seems to be regulated in various ways.

2. Synapse differentiation and signaling

Cadherin-mediated synaptic contacts induce the organization of various molecules at the synapses (FIGURE 8). These include cytoskeletal proteins, scaffold proteins, signaling proteins, proteases, and receptors. Expression of dominant-negative cadherins in hippocampal neurons suppresses the normal recruitment of synapsin and PSD-95 to excitatory synapses (354), and treatment of retinal neurons with blocking antibodies against cadherin 6B impairs the subcellular localization of PSD-95 (136). The N-cadherin-β-catenin complexes bind to an AMPA receptor (GluR2/3) (81, 239), and this association restricts the lateral diffusion of GluR2 (239, 291). Accumulation of GluR4 is blocked by inhibition of cadherins in the retina (342).

Catenins are also important for recruiting postsynaptic components. For example, β-catenin has a PDZ-binding motif and binds to lin-7, S-CAM, and Veli (26, 74, 161, 256). δ-Catenin is expressed specifically in neural tissues, and its localization is restricted to dendrites, indicating an asymmetric localization at synapses (171). δ-Catenin binds to ABP, GRIP, and PSD-95 that interact with GluR2 (314). Interestingly, the molecular composition of cadherin-synaptic molecule complexes changes during synaptic development. For example, β-catenin and p120-catenin are detected in early synapses, but they are replaced by γ-catenin and presenilin during maturation of synapses (287). Furthermore, the cadherin-catenin complexes are involved in intracellular signaling at synapses. For example, N-cadherin mediates IQGAP1/ERK signaling (301). p120-catenin seems to function for maturation of spines through the regulation of Rho-family GTPases signaling as well as that of cadherin levels (83).

Cadherins are also involved in trans-synaptic signaling. Mutant β-catenins with a high affinity for cadherins located in postsynaptic terminals can induce the accumulation of presynaptic molecules and affect synaptic transmission (222). Similarly, chimeric synapses between normal presynaptic terminals and N-cadherin-deficient postsynaptic terminals showed deficiency in presynaptic vesicle exocytosis (155). Since cadherins are homophilic adhesion molecules, their changes in one side of the synapses thus should affect physiology at the other side of them.

3. Synapse transmission

Cadherins are important for neural transmission and its regulation at synapses (FIGURE 8). β-Catenin regulates the localization of synaptic vesicles at presynaptic terminals by binding to cadherins and PDZ-containing proteins such as Veli and scribble (26, 328). N-cadherin, as a partner of β-catenin, is involved in the exocytosis of synaptic vesicles (155). At nascent synapses, N-cadherin and neuroligin-1 cooperate to control vesicle clustering (321). Cadherin-mediated contacts are important for recycling of synaptic vesicles (43, 354). In addition, brain-derived neurotrophic factor (BDNF)-dependent mobility of synaptic vesicles requires the dissociation of cadherin and β-catenin, triggered by phosphorylation of this catenin (25). On the other hand, both localization and stability of AMPA receptors (GluR2) can be regulated by cadherins through their physical associations (239). β-Catenin, which contains a PDZ-binding motif, controls trafficking of AMPA receptors via unidentified PDZ proteins and controls their functions, affecting synaptic strength (245). Moreover, trafficking and positioning of AMPA receptors is controlled by δ-catenin (242). In cadherin-8 knockout mice, physiological coupling between dorsal root ganglion (DRG) neurons and dorsal horn neurons is impaired without overt morphological changes in the synapses (330).

4. Synapse plasticity

Neural activity modulates the number, structure, and efficacy of synapses. In early phases of LTP (E-LTP), rapid modulation occurs without synthesis of proteins, whereas transcriptional regulation and protein synthesis are required for late phases of LTP (L-LTP). Because cadherins are involved in synapse formation and the organization of synaptic molecules as described above, it is natural to consider that cadherins might be part of the machinery responsible for synaptic plasticity. Involvement of cadherins in LTP (E-LTP) was first suggested by experiments in which N-cadherin or E-cadherin is inhibited (345). Interestingly, basal synaptic properties are not affected in this type of experiment. Later reports showed that N-cadherin is important for the induction of L-LTP but not E-LTP (42). In mature synapses of the hippocampus, N-cadherin function is restricted to the stability of coordinated spine enlargement and LTP, and N-cadherin is not required for LTD or the baseline properties of synaptic transmission (44). Cadherin 11 is also involved in the formation of E-LTP (198): in cadherin 11 knockout mice, LTP and the level of LTP saturation are both increased. A recent study showed that N-cadherin is required for mGluR-dependent LTD (403).

Neural activity induces synaptic plasticity via various cadherin pathways (FIGURE 8). Depolarization strengthens N-cadherin-mediated adhesion by its cis-dimerization and induction of a protease resistance (42, 343). It was shown that N-cadherin mediates plasticity-induced long-term spine sta-
bilitation (212). Activation of NMDA receptors (NMDARs) causes a lateral expansion of the spine head and redistribution of cadherins via adhesion-dependent mechanisms (244, 343). Different members of the cadherin family seem to have different characteristics in LTP-associated synapse remodeling (140). Cadherin 8, but not N-cadherin, is selectively removed from remodeled synapses in the middle molecular layer of the dentate gyrus after potentiation. On the

FIGURE 8. Different observations of the roles of cadherins in synapse plasticity. In the steady state, cadherins, localized at the outer rim region of active zone, turn over. After stimulation, more cadherins are accumulated at the synapses, due to suppression of the turnover. This leads to various changes, including an increase in synapse size and the accumulation of presynaptic vesicles, glutamate receptors, and scaffold proteins (top panel). On the contrary, stimulation may release cytoplasmic fragments of cadherins and β-catenin, and these regulate transcription of certain genes (bottom panel). In addition, neural activity may induce destabilization of cadherins. It is necessary to reconcile these different views of stimulation-dependent cadherin actions on synapses.
otherhand, upon activation, translocation of β-catenin to spines occurs, where β-catenin comes into association with cadherins (222). Similarly, neural activity induces the accumulation of αN-catenin at synapses (1). It is notable that αN- and β-catenin are essential for spine stability (1, 245).

Activity-dependent regulation of cadherins may be mediated by the Ca\(^{2+}\) concentration at the synaptic cleft. Extracellular Ca\(^{2+}\) can affect cadherin function directly or indirectly. An interesting hypothesis for the direct regulation of cadherin conformation has been proposed (333): neural activity induces Ca\(^{2+}\) influx via voltage-gated Ca\(^{2+}\) channels and NMDARs, and this action results in depletion of Ca\(^{2+}\) at the synaptic cleft. Cadherin structure and stability strictly depend on the binding of the cadherin to Ca\(^{2+}\) at the EC domains (see sect. IIIA2). Experimental evidence shows that the Ca\(^{2+}\) concentration in the synaptic cleft during stimulation goes down to 0.3–0.8 mM (see Ref. 333 and references therein). This depletion in the Ca\(^{2+}\) level could lead to destabilization of cadherin binding. Thus cadherins could serve as a sensor for neural activity, although this hypothesis is a subject to future testing. In addition, extracellular Ca\(^{2+}\) can regulate cadherin function indirectly. The association between cadherin and AMPARs decreases the lateral diffusion of AMPAR, and this association is regulated by Ca\(^{2+}\) (239, 291). Thus the Ca\(^{2+}\) concentration may regulate AMPAR localization indirectly via its binding to cadherin.

Cadherin function at synapses seems to be regulated by synaptic activity-dependent cadherin turnover. Even under normal, unstimulated conditions, N-cadherin is constitutively turned over via endocytosis at high rates (>40% in 30 min) (334). Upon activation of NMDARs, the rate of N-cadherin endocytosis is significantly reduced via an increase in the binding of N-cadherin to β-catenin (334). Conversely, stabilization of surface N-cadherin blocks NMDAR-dependent synaptic plasticity, indicating a reciprocal dependence between cadherins and plasticity. On the other hand, there is another regulatory mechanism for N-cadherin endocytosis. Following the activation of NMDAR, arcadlin (protocadherin 8) forms a complex with N-cadherin and promotes its endocytosis via the mitogen-activated protein kinase (MAPK) signaling pathway (394) (see below for more details). This arcadlin-mediated endocytosis of N-cadherin occurs after 4 h of activation, and thus this process seems to be a late-onset process to control synaptic function.

Shedding of cadherins from synapses, regulated by proteolytic cleavage, also occurs. Metalloproteinases ADAM10 and MTS-MMP cleave the N-cadherin ectodomain at synapses (216, 280). NMDAR stimulation induces ADAM10-dependent N-cadherin cleavage, and this process is required for subsequent cleavages of N-cadherin by PS1/γ-secretase (364). As a result of proteolysis of N-cadherin, β-catenin is released into the cytoplasm and nucleus, affecting β-catenin signaling (280, 363). Thus ADAM10 regulates synapse structure and function via N-cadherin cleavage (197).

As describe above, there are two contradictory observations on neural activity-dependent cadherin stability: activity induces a reduction in cadherins at synapses via endocytosis and proteolysis, resulting in a temporal destabilization of synapse. On the other hand, neural activity induces cadherin stabilization through the formation of cis-dimers at synapses. This discrepancy remains to be explained.

Cadherins are involved in long-term modifications of synapses via transcriptional control. The sequential cleavages of N-cadherin by ADAM10 and PS1 result in the release of N-cadherin cytoplasmic fragments (N-cad/CTF2) (200, 364), and these fragments induce degradation of CREB-binding proteins that are responsible for transcription of stress-response genes. It is notable that the CREB-dependent gene expression is critical for neural plasticity (12, 189). On the other hand, ADAM10-dependent cleavage of N-cadherin releases β-catenin from the N-cadherin-β-catenin complex into the cytoplasm, which in turn affects β-catenin/LEF-1 transcriptional regulation (280). In addition, NMDAR activation induces the cleavage of β-catenin by calpain (3). The resultant β-catenin fragments promote TCF-dependent gene expression, and the c-fos gene is one of the targets of this transcriptional control. Thus NMDA-R stimulation seems to affect gene expression via proteolysis of cadherins and catenins.

Through the activity-dependent modulation of synaptic structure and efficacy, cadherins are likely involved in high-order neural activities, such as learning and memory. For example, N-cadherin is thought to be involved in the formation of long-term contextual fear memory via IQGAP/Erk1/2 signaling (301). Cadherin 11 is important for formation of fear- or anxiety-related responses (198). β-Catenin in the amygdala appears to be required for memory consolidation (194). αN-catenin mutants also show abnormal startle responses in addition to ataxia (252). In addition, deletion of δ-catenin leads to impairment of visually driven cortical responses and severe cognitive dysfunction (149, 207). These physiological functions of cadherins are likely linked to psychiatric disorders, and their relationship will be described in the next section.

E. Cadherin Deficiency Related to Brain Diseases

Psychiatric disorders such as schizophrenia and mood disorders are often multifactorial, and they show complex symptoms, many of which are shared by different neural disorders, thus suggesting the involvement of various genes. Therefore, the identification of causative genes and/or susceptibility genes is difficult and has been delayed. Because the cadherin-catenin complexes are involved not only in
developmental steps but also in synapse formation and functions, it is not surprising that some defects in the cadherin-catenin system impair high-order neural functions. Up to now, certain cadherin or catenin genes have been assigned as major genes causative of specific diseases (see also review in Ref. 82). Furthermore, recent advancements in genome-wide analysis are making it possible to identify various cadherin/catenin-related genes as susceptibility genes or risk factors for particular neural disorders (96).

The cadherin and catenin loci that are associated with neural disorders are summarized in Table 4. For example, deletion of the CDH15 (Cadherin 15, M-cadherin) gene results in mental retardation (intellectual disability) (33), and hemizygous loss of the δ-catenin gene is associated with severe mental retardation phenotypes of cri-du-chat syndrome (211). In addition, single nucleotide polymorphisms (SNPs) in the intergenic region between the CDH9 and CDH10 genes are associated with autism spectrum disorder (ASD) (378), and deletion of the region between the CDH12 and CDH18 genes is linked with schizophrenia (316). The αN-catenin locus is associated with addiction-related phenotypes, schizophrenia, and bipolar disorder (63, 185, 186, 367). Defective N-cadherin genes were identified as a candidate for canine compulsive disorders (CCDs) (75).

Molecular mechanisms underlying neural disorders in which cadherins are involved are elusive, although dysfunction of cadherins is likely the main cause. In some mental retardation patients, four nonsynonymous variants in the NH2-terminal region of M-cadherin have been identified. Interestingly, three of these M-cadherin variants show reduced cell-adhesion promoting abilities (33). Mutant M-cadherins may cause mental retardation by reducing neural transmission at synapses, because M-cadherin is known to be localized at synapses, at least in the cerebellar glomeruli (286). In contrast, many mutations and variations have been identified in the noncoding regions, i.e., in the intergenic regions or introns. These mutations/ variations possibly cause aberrant gene expressions, but how these genetic loci cause or affect the diseases remains to be explained (56, 316).

IV. NONCLASSICAL CADHERINS

A. Protocadherins

Protocadherins constitute the largest group in the cadherin superfamily. In humans, out of ~110 cadherin superfamily members, 64 are protocadherins (387, 388) (Table 1). Protocadherins (Pcdhs) are divided into two groups according to their genomic organization. The first group is called “clustered protocadherins,” whose genes are located in tandem arrays on chromosome 5 in the human genome (Figure 9). Three gene clusters (Pcdhα, Pcdhβ, and Pcdhγ) have been identified, which encode more than 50 members in total. The second group contains diverse members, and their genes are scattered over different chromosomes. These “nonclustered protocadherins” are further divided into δ-protocadherins (δ1- and δ2-protocadherins) and others (371). Protocadherins are not specific to vertebrates, but they have rapidly evolved in the vertebrate lineage (139). Sequence analysis suggests that protocadherins are derived from Fat-like molecules (see below) and that the six repeat-type protocadherins are derived from the seven-repeat type by deletion.

Protocadherins are expressed in the nervous system (169, 295). Most of them can enhance cell aggregation in a homophilic fashion (e.g., Pcdh2 to Pcdh2, and Pcdh3 to Pcdh3), when they are expressed in cells. However, the protocadherin-mediated cell-cell adhesions are generally weak, compared with the classical cadherin-mediated ones (295). Adhesion mechanisms based on protocadherins seem to be slightly different from those for classical cadherins (218). In the case of Pcdh4, the overall topology of its EC1 domain is similar to that in the classical cadherins. However, Trp2, the residue important for “strand swap” in classical cadherins, is not conserved in the protocadherins, and the hydrophobic pocket for interacting with Trp2 is not as deep as that in the classical cadherins. Moreover, recent studies suggest that EC2 and EC3 are important for the homophilic bindings between Pcdhγ molecules rather than EC1, which is critical for those between the classical cadherins (300). The cytoplasmic domains of protocadherins diverge among the members, suggesting that these molecules may have diverse signaling functions.

Because of their diversity and expression in the brain, protocadherins have been speculated to be involved in generating neural diversity for neuronal differentiation and synapse formation (388). Although the functions of protocadherins remain mostly unknown, we have summarized recent progress in the studies on these molecules.

1. Clustered protocadherins: protocadherin α, β, and γ

The clustered protocadherin family consists of three subfamilies or clusters: protocadherin α (Pcdhα, CNR), protocadherin β (Pcdhβ), and protocadherin γ (Pcdhγ) (387) (Figure 9). In each cluster in humans, there are more than 10 genes. In the case of Pcdhα and Pcdhβ subfamilies, each gene uses an independent promoter and all the genes share a common exon encoding the constant COOH-terminal region by alternative splicing, whereas the Pcdhβ cluster seems to lack the constant exon (349, 379, 387). Because their genomic organizations are similar to those for immunoglobulin genes, the generation of diversity has been expected. However, extensive studies indicate that genetic rearrangement, somatic mutation, and trans-splicing generally do not occur in the expression of Pcdhα genes (388). Individual neurons show the monoallelic and combinatorial expression of each exon of the Pcdhα genes (89). Recent
<table>
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<th>Diseases</th>
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<td>Addiction</td>
<td>Uhl et al. (367)</td>
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</table>

This is not a complete list. ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; DFNB, nonsyndromic hearing loss and deafness; BPAD, bipolar affective disorder; EFMR, X-chromosome-linked epilepsy and mental retardation limited to females. *Reference is a review.
in vitro studies suggest that the trans-interaction between Pcdhγ isoforms is strictly homophilic, whereas they nonselectively cis-interact with other isoforms (300). Furthermore, Pcdhα forms heterodimers with Pcdhγ presumably in a cis fashion (223). Therefore, the combinational diversity of protocadherin cis-dimers could be enormous, and protocadherins can thus give rise to more diversity in their binding specificity than classical cadherins.

In neural tissues, Pcdhγs are localized at synapses (92, 95, 187, 261), and their deficiency results in a reduction in not only synapse number but also synapse size (379, 382). Thus this group of protocadherins might be required for synapse maturation. Partly consistent with this view, experiments using cocultures of neurons and astrocytes obtained from wild and/or mutant mice suggest that Pcdhγs support synaptogenesis by promoting astrocyte-neuron contacts, although their expression in neurons is required for synapse maturation (103). Pcdhγs are also involved in cell survival. Deletion of this gene cluster leads to apoptosis of spinal interneurons, retinal interneurons, and retinal ganglion cells (182, 379). However, the mechanisms for this cell survival seem to be different between the spinal cord and retina. In the spinal cord, cell survival depends on synapse formation (i.e., non-cell-autonomous), whereas, in the retina, it occurs in a cell-autonomous manner (182, 265). Precise roles of Pcdhγs in cell survival have not been determined yet, although there is a suggestion that they might be involved in transcriptional control. Pcdhγ is processed by presenilin, and the resultant cytoplasmic domains induce Pcdhγ expression (109, 111). In addition, it is reported that Pcdhγ-B1 interacts with a microtubule-destabilizing protein, SCG10, but the role of this interaction is unknown (103).

Pcdhαs (CNRs) are expressed in the olfactory bulb, cerebellum, and hippocampus. Pcdhαs are also detected at synapses (37, 169). Recent studies on Pcdhα knockout mice show that Pcdhα is involved in the axonal coalescence of olfactory neurons into glomeruli (117), and in serotonergic projections (159). Moreover, downregulation of Pcdhα isoforms leads to learning abnormalities (99). On the other hand, the molecular properties of Pcdhα are not fully understood. It was reported that Fyn, neurofilament, and fascin bind to the cytoplasmic region of CNR but that their in vivo interactions are not clear (169, 359, 388). A recent study shows that Pcdhα interacts with Ret (297).

Pcdhβ (Protocadherin 3) also constitutes a gene family, but this gene cluster does not have constant exons. These molecules have a weak adhesion activity in vitro (292). Pcdhβ16 was shown to be localized at postsynapses (154), but its role there remains unknown. Overall, the clustered protocadherin family likely plays important roles in neural development and function. Further studies are, however, required to determine their roles in the brain.

2. Nonclustered protocadherins: OL-protocadherin (protocadherin 10)

OL-protocadherin is a member of the δ2-protocadherins and shows a weak adhesion-promoting activity when cell lines are transfected with it (130). This protocadherin was suggested to be a tumor suppressor gene in carcinoma cells (396). In the nervous system, OL-protocadherin is expressed in various regions during development, and its expression pattern has some correlations with certain neural networks such as the olfactory and limbic systems (15, 130, 231). However, the defects observed in OL-protocadherin knockout mice are restricted to specific neural projections.
in the ventral telencephalon, including thalamocortical and corticofugal projections (365) (FIGURE 10A). Close analysis of these mice suggests that OL-protocadherin is involved in axon outgrowth from the striatum (365) (FIGURE 10B) and that the proper growth of striatal axons is required for the subsequent neural circuit formation in the ventral telencephalon (126, 365).

Cell biological studies have been revealing the molecular roles of OL-protocadherin in cell-cell contacts (FIGURE 10C).
When OL-protocadherin is expressed in cells, it enhances their movement within the colonies. The cytoplasmic domain of OL-protocadherin binds Nap-1, a component of the WAVE complex, which regulates actin polymerization (230). As a consequence, OL-protocadherin recruits the WAVE complexes to cell-cell contacts. It has been proposed that this WAVE complex induces rearrangement of actin filaments, and in turn destabilizes the strong adhesion mediated by classical cadherins, resulting in the promotion of cell movement. If this molecular event occurs between axons, OL-protocadherin might facilitate the outgrowth of stratal axons, although this model needs to be tested in the future study. A recent study showed that one of the autism loci is located near the OL-protocadherin gene (TABLE 4) (220).

3. Protocadherin 8 (Arcadlin) and paraxial protocadherin

Arcadlin/protocadherin 8 was identified as a molecule induced by neural activity (389), and it shows a homophilic adhesion activity. Although protocadherin 8 has been suggested to regulate somitegenesis (281), its knockout mice do not show any visible defects during embryogenesis (391).

In neural tissues, arcadlin is localized at synapses, and antibodies against arcadlin reduce excitatory postsynaptic potential (EPSP) amplitude and block LTP, suggesting its involvement in synaptic plasticity (389). Recently, part of the arcadlin signaling pathway has been disclosed (394); neural activity induces the activation of arcadlin signaling via TAO2b and p38MAP kinases, resulting in endocytosis of N-cadherin. This reduction in synaptic N-cadherin seems to lead to a reduced spine number. Neural activity-induced spine loss appears to be contradictory to induction of LTP. However, the arcadlin-induced N-cadherin endocytosis occurs only after 4 h, and the authors speculated that the arcadlin-mediated N-cadherin endocytosis may provide a homeostatic mechanism to control neural complexity. It is possible that spine loss after epilepsy may be explained by this arcadlin-induced signaling and shedding of N-cadherin (327). Arcadlin also binds to cadherin 11, suggesting that it also regulates cadherin 11 endocytosis (394).

Paraxial protocadherin (PAPC) in amphibians and zebrafish is closely related to protocadherin-8, but it may not be an actual ortholog of protocadherin 8, but rather an amphibian-specific member (138). This molecule is highly expressed in the paraxial mesoderm, and its expression is regulated by various molecules and signaling pathways including the spadetail transcription factor, transforming growth factor (TGF)-β signaling, β-catenin signaling, and Wnt-5A/Ror2 signaling (53, 298, 383, 390). It has been shown that PAPC is involved in convergence and extension movements during gastrulation via two independent pathways, i.e., the Frazzled-7 PCP signaling pathway and C-cadherin-mediated adhesion (53, 167, 369, 380). In addition, ankyrin repeats-domain protein-5 (ANR5) binds to the cytoplasmic domain of PAPC and regulates gastrulation (58). Moreover, PAPC forms a complex with FLRT3 and C-cadherin, and PAPC controls C-cadherin-mediated adhesion by regulating FRT3 activity via GTPase RND1 (54). Oligomerization of PAPC by disulfide bonding is needed for its cell sorting function via C-cadherin (55). PAPC is also involved in somitegenesis (166).

4. Protocadherin 19

Protocadherin 19 (Pcdh19) is also one of the δ2-cadherins. Pcdh19 is mainly expressed in the central nervous system and retina (335). It has been reported that Pcdh19 interacts with N-cadherin and that these two molecules act synergistically in cell movements during neurulation (34). In zebrafish, dysfunction of Pcdh19 leads to abnormal brain morphogenesis as a result of arrest of cell convergence in the anterior neural plate (85).

In humans, mutations in Pcdh19 have been implicated in an X chromosome-linked epilepsy and mental retardation limited to females (EFMR), as well as in Dravet-like syndrome (69, 73, 201) (TABLE 4). It has been speculated that the male is unaffected by compensation with nonparalogous PCDH11Y, whereas the female is affected as a result of the “cellular interference” by mosaicism via X-chromosome inactivation (73). The importance of mosaicism in the development of Dravet-like syndrome is supported by the existence of mosaic males who have de novo mutations and show epileptic encephalopathy (69).

5. Other protocadherins

Other protocadherins are also expressed in the nervous system, but studies on these molecules are still in progress. Protocadherin 7 (NF-protocadherin, BH-protocadherin) has been shown to be important in formation of the embryonic ectoderm (45). This protocadherin interacts with TAF1, and both of these molecules are required for neural tube formation (272) and also for retinal axon initiation and elongation (262). Protocadherin 7 is also known to interact with protein phosphatase 1a (398).

B. Cadherin 23 and Protocadherin 15 in Inner Ear Functions

Cadherin 23 (Cdhh23) and protocadherin 15 (Pcdh15) are large cadherins with 27 and 11 cadherin repeats (EC domains), respectively. These molecules are distantly but evolutionarily related to one another with a low sequence homology in their cytoplasmic regions, although the nomenclature is confusing (138). These cadherins are expressed in various regions: Cdhh23 is expressed in the testis, skeletal muscle, heart, and eye, in addition to the brain, whereas Pcdh15 is expressed in the sensory epithelia of the cochlea,
The cochlea in the inner ear is responsible for hearing. Sound evokes vibration of the endolymph in the cochlea. Hair cells of the Corti’s organ sense these vibrations and transform the mechanical vibration into neuronal signals. There are two types of hair cells: outer hair cells (OHCs) and inner hair cells (IHCs). Both hair cells have hair bundles for the detection of vibration, where the OHCs are responsible for the amplification of the sound-evoked vibration, whereas the IHCs are real sensory cells that are responsible for the mechanoelectrical transduction.

Proper development of the hair bundles is important for mechanotransduction. The hair bundles of IHCs are V-shaped arrays of actin-filled stereocilia, which are connected with one another with special structures named tip links. In early development, hair bundles are formed from many stereocilia and one specialized cilium, named the kinocilium (FIGURE 11A). This kinocilium is considered to be important in the formation of stereocilia bundles through the control of planar cell polarity. In early developmental stages, stereocilia and the kinocilium are transiently linked.
CADHERINS IN BRAIN

Cdh23 and Pcdh15 are involved in the formation of hair bundles (FIGURE 11A) (183, 221, 258, 302). Both undergo heterophilic interactions so as to organize the lateral links and kinociliary links (107). In either Cdh23 or Pcdh15 knockout mice, hair bundles are fragmented and misoriented, and displacement of the kinocilium is observed. On the other hand, the ankle links do not consist of cadherins but of VLGR1-Usherin-Vezatin complexes [see Müller (221) and references therein].

Cdh23 and Pcdh15 are also involved in mechanotransduction in mature hair cells. Both molecules are found in the top connectors and tip links in mature hair bundles. The Cdh23 dimer is localized at the upper part of a tip link, whereas the Pcdh15 one is located at the lower part of the same tip link (163). Parallel homodimers of Cdh23 interact in trans with Pcdh15 homodimers in a Ca\(^{2+}\)-dependent manner (163, 221). It has been reported that the length of tip links varies between 90 and 190 nm depending on the Ca\(^{2+}\) concentration (21, 100). There seems to be more components in the tip links, because tip links still can be seen in Cdh23 knockout mice (288). The details of the molecular composition of the tip links thus remain to be further analyzed.

The mechanism for the heterophilic interaction between Cdh23 and Pcdh15 is somewhat different from the strand-swap binding of classical cadherins (84). The NH\(_2\) terminus of Cdh23 contains polar amino acids that bind Ca\(^{2+}\), and this Ca\(^{2+}\) binding is critical for the interaction of Cdh23 with Pcdh15. The NH\(_2\)-terminal region of Cdh23 is extended to form this Ca\(^{2+}\) binding site, and a deafness mutation in this region reduces Ca\(^{2+}\) affinity and rigidity of the molecule (320).

Cdh23 and Pcdh15 interact with cytoskeletal elements that are also causative of Usher syndrome 1 (221). The cytoplasmic region of Cdh23 (USH1D) interacts with harmonin-\(\beta\) (USH1C) and myosin 1c, whereas that of Pcdh15 (USH1F) interacts with harmonin-\(\beta\) and myosin 7a (USH1B). Harmonin is an adaptor protein with three PDZ domains, and it also interacts with F-actin and another adaptor protein, Sans (USH1G), suggesting this molecule to be involved in cytoskeleton assembly. On the other hand, myosin 7a and Sans are required for transportation of harmonin-\(\beta\) into stereocilia (82). Moreover, the mechanotransducer channel, the structure of which has not been determined yet, is localized at Pcdh15 side (i.e., lower part) of the tip links, and myosin 7a may be involved in gating mechanotransduction channels (32, 134, 174). Although the function of cytoplasmic interactors for Cdh23 and Pcdh15 is not well understood yet, it is clear that these molecules are critical components of the machinery for hair bundle cohesion and mechanotransduction.

There are several splice variants of Cdh23 and Pcdh15 (120, 177). Cdh23 has three major isoforms, including the 27-EC domain type (type a), the 7-EC domain type (type b), and the cytoplasmic domain-only type (type c). There is another minor variation, one that lacks the 68th exon. Interestingly, Cdh23a+68 is expressed only in hair cells, whereas Cdh23a-68 is expressed in the retina. Pcdh15 exists as four major isoforms named CD1, CD2, CD3, and SI. CD1, CD2, and CD3 have 11 EC domains but have different cytoplasmic domains, and SI lacks the transmembrane and cytoplasmic domains (6). These isoforms are localized differentially along hair bundles: CD2 is found in the early transient lateral links and CD3 in the tip links. The functional differences among these isoforms remain to be determined.

C. Celsr/Flamingo in Brain Morphogenesis

Celsr/Flamingo is a unique group of cadherins with a seven-pass transmembrane domain (110, 370). The extracellular domain contains nine EC domains, several EGF-like repeats, and two laminin globular (LAG) domains. These molecules undergo homophilic interactions, as do other cadherins, but their heterophilic interaction with unknown ligands has also been postulated (168, 309, 370). Originally, the molecules named Celsr and Flamingo (Fmi) were discovered independently; Celsr was found in mammals (110), whereas Fmi/Starry night (Stan) was identified in Drosophila (370). Accordingly, “Celsr” is generally used for the vertebrate molecules and Fmi, for the invertebrate homolog. In mammals, there are three Celsrs: Celsr1 (Fmi2), Celsr2, and Celsr 3 (Fmi1).

A major function of Celsr/Flamingo is the regulation of planar cell polarity (PCP), with this molecule acting as a component of the core frizzled (Fz)/PCP gene groups (FIGURE 12) (315, 374). In Drosophila wings, Fmi is localized asymmetrically in individual cells at their contact sites and regulates PCP signals by interacting with Frizzled and Van Gogh (Vang)/strabismus (Stbm). Importantly, the Fz/PCP signaling pathway is highly conserved across species and tissues. In vertebrates, typical PCP phenotypes can be seen in the patterning of cochlear stereocilia and of skin hair, and Celsr1 is involved in this patterning (64, 274). In vertebrate neural tissues, the convergent extension of neuroepithelial cells is con-
Celsrs have also been suggested to control neuronal morphogenesis via PCP-independent pathways (102, 168). In mammals, Celsr2 is involved in dendritic growth, and also in the maintenance of dendritic branches (310). Interestingly, Celsrs control neurite growth via multiple signaling pathways: Celsr2 enhances neurite growth possibly via CaMKII, whereas Celsr3 suppresses it via calcineurin (309). In the mouse forebrain, Celsr3 sustains interneuronal migration by regulating the expression of neuregulin-1 and its receptor, ErbB4 (395). Thus various signaling pathways, including the Fz/PCP pathway, are involved in Celsr-mediated neuronal morphogenesis and axon guidance.

D. Fat and Dachsous in Brain Morphogenesis

Fat cadherin has 34 EC domains, EGF repeats, and 2 LAG domains (FIGURE 1) (318, 348). Fat molecules heterophilically interact with another member of the cadherin superfamily, Dachsous (Ds) (61, 203). Dachsous is also a large molecule with 27 cadherin motifs. fat and dachsous genes were cloned originally as tumor suppressor genes in Drosophila, and many studies on these molecules have been carried out in the Drosophila system (61, 195, 318). Fat and Dachsous control cell prolifer-
tion via the Hippo pathway, and also regulate planar cell polarity (see reviews in Refs. 180, 318). *Drosophila* has another Fat-like cadherin, Fat2, but this molecule is not responsible for growth and planar cell polarity, but is important for tubule formation (50). In the mammalian genome, there are four Fat-related genes and two Dachsous genes (TABLE 5) (318, 348). Among them, Fat4 is considered to be an ortholog of *Drosophila* Fat, whereas Fat1–3 belong to a different subfamily. These mammalian Fat and Dachsous genes are expressed differentially in the nervous system during development, as well as in the adult (285). However, the studies on Fat and Dachsous cadherins in the mammalian nervous system are just beginning.

Mammalian Fat4 is involved in PCP including the orientation of hair cells in the inner ear and neural tube elongation (289). However, no abnormalities in the proliferation are evident in Fat4-deficient mice. Recent studies show that Fat4 and Dachsous1 are essential for organizing the apical membrane in the embryonic cerebral cortex (148). In this region, Fat4 forms a complex with MUPP1 and Pals1, which seems to mediate some signals required for maintaining the apposition of apical membranes.

On the other hand, mammalian Fat1 is involved in cell-cell contacts and lamellipodial dynamics through the control of the actin cytoskeleton via Ena/VASP (347). Fat1-deficient mice have abnormalities in their renal slit junctions, and defects in forebrain and eye development (59). Fat2 is specifically expressed in cerebellar granule cells and localized on parallel fibers (232). Fat3 is expressed in various regions of the mouse nervous system, and it seems to be involved in interactions between neurites during development (226).

### E. Calsyntenin/Alcadein

Calsyntenin/alcadein is a group of type 1 transmembrane proteins with two cadherin repeats in their extracellular domain (18, 124, 375). Calsyntenins are abundant in axons in postnatal neurons, whereas they are localized at postsynaptic membranes in adult neurons (170). It remains unknown whether these molecules undergo homophilic interactions or heterophilic interaction with other molecules.

Calsyntenins are involved in kinesin-dependent vesicle transport (FIGURE 13). When the extracellular domain of calsyntenin-1 is cleaved by proteases, the membrane stump is internalized and accumulates in the spine (375). The cytoplasmic region of calsyntenin-1 binds to the kinesin light chain and functions as a novel cargo-docking protein in kinesin-mediated transport (16, 170). A recent study showed that calsyntenin-1 is involved in distinct vesicle transport systems including long-recycling endosomal vesicles and early-recycling ones (325). Thus calsyntenins may play a role in various neural phenomena via the control of vesicle trafficking.

Moreover, calsyntenins seem to be involved in amyloid precursor protein (APP) metabolism, at least in part. On the cell surface, calsyntenin binds APP via X11-like protein, forming a stable tripartite complex (17, 18). Dissociation of the X11-like protein from the complex induces cleavage of APP and calsyntenin, generating fragments of

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**Table 5. Summary of Fat and Dachsous cadherins**

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<td>Cell growth</td>
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<td>Dachsous 1</td>
<td>Similar to Fat4 mutant phenotypes</td>
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<tr>
<td>Dachsous 2</td>
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</table>

| Drosophila     |                                                                                  |                |
| Fat           | Growth and PCP regulation                                                        | Sopko and McNeill (318)* |
| Fat-like      | Tubular formation                                                                | Castillejo-Lopez et al. (50) |
| Dachsous      | Cell growth and morphogenesis                                                     | Clark et al. (61) |

*Reference is a review.
both proteins. It has been shown that calsyntenins recruit kinesin-1 to the trans-Golgi network and mediate the export of APP from the trans-Golgi network (190). Interestingly, the inhibition of the transport of APP-containing vesicles increases the generation of amyloid β-protein (Aβ) and perturbs neuronal activity in Drosophila (16).

Calsyntenins seem to be closely related to high-order neural functions. It has been reported that calsyntenins are involved in learning and memory in both Caenorhabditis elegans and humans (143, 249). Interestingly, calsyntenin function has been highly conserved during evolution, and human calsyntenin-2 can rescue associative learning phenotypes in C. elegans calsyntenin-1 mutants (132). The ectodomain of calsyntenin is required for learning (143). Although it remains unknown how calsyntenin regulates memory formation, it may be involved in regulation of glutamate signaling pathways (132).

F. Other Cadherins

1. T-cadherin (cadherin-13, H-cadherin)

T-cadherin is a unique GPI-anchored cadherin that lacks the cytoplasmic domain. Despite this unique structure, T-cadherin can mediate homophilic adhesions in vitro (290). Its binding mechanism is somewhat different from that of classical cadherins: T-cadherins form trans-dimers at the nonswapped interface near the EC1-EC2 calcium binding sites (60). T-cadherin may function as a signaling receptor in the regulation of cell motility and proliferation in vivo (260). However, our knowledge about its function in the nervous tissue is rather fragmentary.

Trunk neural crest cells and motor axons migrate within the rostral half of each sclerotome. T-cadherin is expressed in the caudal half of the somitic sclerotome, suggesting a repulsive influence on the migration of neural crest cells and motor axons (271). In fact, T-cadherin inhibits the growth of motor axons in vitro (97). However, T-cadherin is expressed in the growing motor axons, but not in neural crest
cells, leaving the role of T-cadherin in neural crest cell migration unexplained (271). T-cadherin is redistributed from cell-cell contact sites to growth cones upon application of nerve growth factor (24). Recently, T-cadherin has been identified as a molecule that acts as a positive regulator of synapse formation (250).

2. Protocadherin 21 (photoreceptor cadherin, PrCAD)

Another interesting cadherin is protocadherin 21 (PrCAD), a cadherin specifically expressed in the outer segment of photoreceptor cells of the retina (273). This cadherin has six EC domains and a unique cytoplasmic domain. Despite its nomenclature, protocadherin 21 is more similar to cadherin 23 and protocadherin 15 than to the authentic protocadherins (138). In its knockout mice, the outer segments are disorganized, and photoreceptor cells progressively die (273). Recently, this gene was shown to be responsible for human recessive retinal dystrophies (Table 4) (121, 246).

V. CONCLUSIONS AND FUTURE PERSPECTIVES

In the past 20 years, the studies on classical cadherins in neural development have greatly progressed. Various roles of classical cadherins in neural tissue morphogenesis have been proven by a variety of experimental approaches. Their roles in synapse formation and function are also being unveiled. Importantly, as synaptic functions are closely associated with high-order neuronal functions, dysfunction of cadherins likely results in brain disorders. On the other hand, at the molecular level, evidence has been accumulated that the cadherin-catenin system is linked to and orchestrated with other diverse cellular systems. Moreover, most members of the cadherin superfamily show biological functions distinct from those of classical cadherins. Therefore, the cellular phenomena controlled by cadherins are diverse and complex.

Despite much progress in the field of cadherin research, many important issues remain to be resolved. For example, the specific role of each classical cadherin subtype in brain functions is largely unknown. Many of the classical cadherin gene knockout mice do not show any apparent developmental abnormalities. Although these animals may have physiological defects, it is not always easy to identify such defects in mice. It is also highly probable that multiple cadherin subtypes have redundant functions, which may result in hindering the effect of removal of a single cadherin subtype in knockout mice. Indeed, each neuron expresses multiple cadherin subtypes in a unique combination. Systematic genetic studies are therefore necessary to solve this problem.

Another related issue, which should be further pursued, is to verify the in vivo roles of the cadherin specificities. In the interactions between classical cadherins, they choose specific partners. This nature of cadherin interaction can well explain many cell-sorting phenomena or axonal target recognition processes (275, 338). Protocadherins also have strict specificities in their binding (300). Thus cadherins have the potential to create enormous varieties of combinatorial specificities for cell-cell interactions. On the other hand, in Drosophila, DN-cadherin is used for axonal target recognition, and this is the only cadherin expressed in the Drosophila nervous system, except for its isoform. In such cases, the spatiotemporal regulation of cadherin functions, rather than specificity, appears to work in cell-recognition processes. Cadherin regulation can be achieved via its interactions with other molecules, or more indirectly via transcription factors.

The physiological roles of classical cadherins and other members of the cadherin superfamily in synapses are still poorly understood. Most studies on the synaptic cadherins have thus far been carried out by using cultured neurons, leaving their in vivo roles mostly undetermined, except for a few examples. Human genomic studies are unveiling cadherin genes involved in brain dysfunction such as psychiatric diseases. Such information should be helpful for deeper analysis of cadherin functions in the human brain.

In this review, we described various members of the cadherin superfamily one by one. What is common among these molecules is that all of them, except for the uncharacterized ones, undergo homophilic or heterophilic interaction through their extracellular cadherin-specific domains. However, the amino acid sequences of their cytoplasmic domains are quite diverse. Thus, although cadherins are grouped into a single superfamily based on the similarities in the extracellular domains, they produce distinct signals through their family- or subfamily-specific cytoplasmic domains. Consequently, the cadherin superfamily regulates many different behaviors of cells, such as adhesion, proliferation, and planar cell polarity. To understand how this diversity in cadherin molecules has evolved is another interesting subject in the cadherin field.

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Addresses for reprint requests and other correspondence: M. Takeichi, Laboratory of Cell Adhesion and Tissue Patterning, RIKEN Center for Developmental Biology, 2–2–3 Minatojima-Minamimachi, Chuo-ku, Kobe 650–0047, Japan (e-mail: takeichi@cdb.riken.jp); and S. Hirano, Dept. of Neurobiology and Anatomy, Kochi Medical School, Okoh-cho
Kohasu, Nankoku-City 783–805, Japan (e-mail: s-hirano @kochi-u.ac.jp).

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DISCLOSURES

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SHINJI HIRANO AND MASATOSHI TAKEICHI


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CADHERINS IN BRAIN


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**CADHERINS IN BRAIN**


ensemble of cell-cell-attachment characteristic for transformed mesenchymal cells. 


Shinji Hirano and Masatoshi Takeichi


