Neural development: **How cadherins zipper up neural circuits**
Sebastian Martinek and Ulrike Gaul

Recent studies suggest that members of the cadherin family of homophilic cell adhesion molecules play an important role in the formation and stabilization of the complex neural circuitry of the brain.

Address: Laboratory of Developmental Neurogenetics, Rockefeller University, 1230 York Avenue, New York, New York 10021, USA. E-mail: martins@rockvax.rockefeller.edu and gaul@rockvax.rockefeller.edu

The functioning of the nervous system depends on precise patterns of connections between an enormous number of neurons [1]. How the complex neural architecture of the brain is generated and how subsequently the extraordinarily precise patterns of neuronal connections are established during development is still poorly understood. Newly born neurons first migrate to their final location and then often organize themselves into nuclear or laminar structures. Upon reaching their final destination, neurons usually begin to form extensions — axons, which may grow over long distances to connect with their targets. With astonishing accuracy, axons manage to find their path in often very complex territory and to recognize their specific target among a myriad other options. The growing tip of the axon, the growth cone, is thought to detect guidance cues in the environment and thereby steer the movement of the axon. When they reach their target, the axons establish specialized contacts — the synapses through which neurons communicate with their target cells — eventually forming functional neural circuits.

One group of molecules considered to be important for neural circuit formation is the cadherin family, cell-surface glycoproteins that mediate calcium-dependent cell adhesion [2,3]. Cadherins are thought primarily to engage in homophilic interactions, though in several cases weaker, heterophilic interactions have been reported [4–6]. On the basis of their sequences and functions, cadherins have been largely subdivided into classical, desmosomal and proto-cadherin classes, some members remaining unclassified [2,7]. All cadherins have in common a distinctive, tandemly repeated sequence motif in their extracellular segment [2,8]. Analysis of the three-dimensional structure of this extracellular domain suggests that cadherins form lateral dimers within the plasma membrane [9]. Each lateral dimer is thought to be linked to two equivalent dimers on the opposite cell surface, thus forming a supermolecular structure that has been called a 'cell-adhesion zipper' [8,9] (Figure 1). Most classical cadherins have a transmembrane domain and a highly conserved intracellular domain, which can be linked to the actin cytoskeleton *via* a complex of adaptor proteins, including α-catenin, β-catenin, plakoglobin and p120^{cas} [10].

Cadherin-mediated cell adhesion appears to be regulated at a number of different levels. The type and the amount of cadherin expressed is highly cell-type specific [11]. The intracellular association of cadherins with the cytoskeleton is thought to be controlled through phosphorylation of the cadherin–catenin complex [10,12]. Cadherin function has mostly been studied *in vitro*. These experiments have shown that, as a consequence of preferential homophilic binding, cells expressing the same cadherin aggregate with each other and segregate from cells expressing other types of cadherins [2]. Furthermore, cells expressing the same cadherin in different amounts also segregate [13]. These findings led to the suggestion that, in a similar fashion, differential expression of cadherins *in vivo* could result in

Figure 1
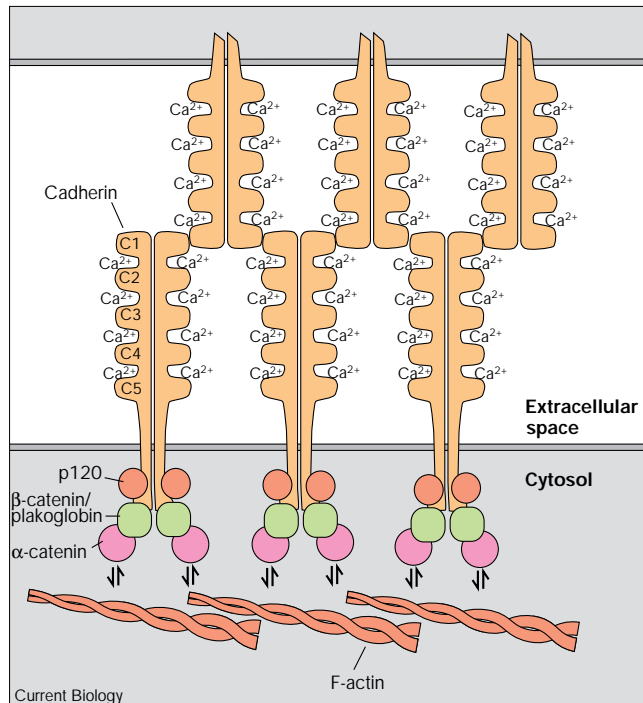
The 'cell-adhesion zipper' superstructure of cadherin–catenin complexes [10]. The complexes are thought to be linked to the actin cytoskeleton inside the cell, but the precise nature of the link is not known. C1–C5, cadherin domains 1–5; p120, cadherin-associated Src-substrate.

Figure 2

N-cadherin plays a part in the layer-specific arborization of retinal axons in chick optic tectum [23]. **(a)** In a retinotectal coculture, retinal axons normally extend along the stratum opticum (SO) and form rudimentary arbors in the three specific tectal layers (RL) that receive retinal input. **(b)** In the presence of anti-N-cadherin antibodies, the retinal arbors are simplified and some neurites extend beyond the tectal layers where they normally terminate.
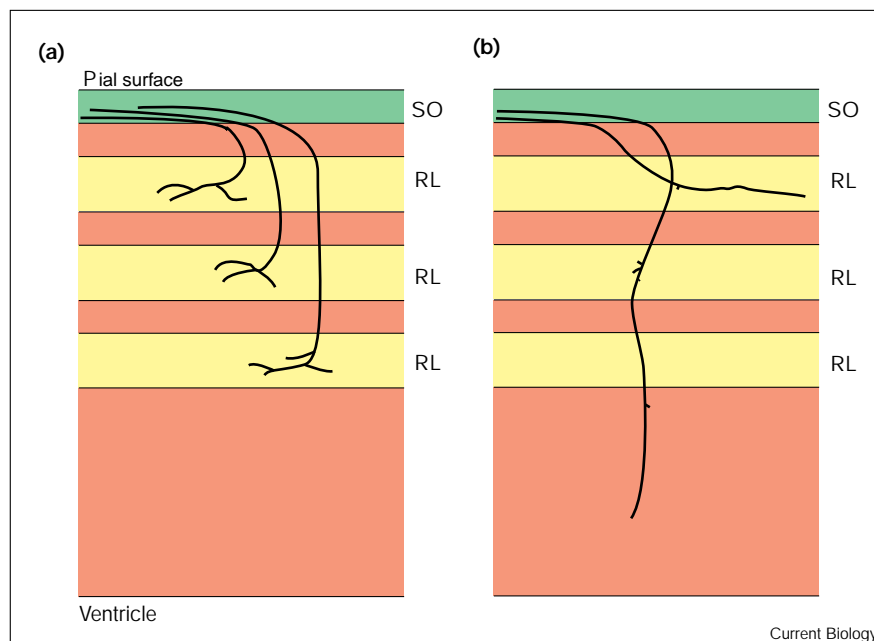
differential adhesiveness of cells, which in turn could drive morphogenetic processes during development.

Cadherin proteins have been found to be expressed in highly dynamic and specific patterns throughout the development of the vertebrate nervous system [7,11]. In early development, various cadherins are regionally expressed in the brain, delineating specific neuromeric subdivisions or boundaries. At later developmental stages, cadherins begin to be expressed in a restricted set of brain nuclei. These localization patterns suggest that cadherin-mediated adhesion may play a role in the establishment or maintenance of the cytoarchitecture of the central nervous system (CNS).

Later in development, many cadherins begin to be expressed in developing nerve fiber tracts. Cadherin expression by neurites generally appears to be particularly strong at the time of axon elongation and tract formation, and to diminish once the neurites have reached their target [11]. As at least N-cadherin and R-cadherin have been shown to promote neurite outgrowth *in vitro* [14–16], cadherins may serve as homophilic guidance molecules for the navigation of neurites along pre-existing fibers expressing identical cadherins, thereby promoting the selective bundling of nerve fibres into fascicles (selective fasciculation). Strong evidence for a role of cadherins in axon fasciculation has come from a recent genetic study of the novel *Drosophila* N-cadherin [17].

In the fruitfly, three cadherins were already known: DE-cadherin, a classical cadherin, which had been shown to associate with the *Drosophila* catenin homologs Dα-catenin

and Armadillo (β-catenin) [18]; and two other, unclassified members of the family, Fat and Dachsous [19,20]. None of these *Drosophila* molecules is primarily expressed in the nervous system. While having diverged from classical vertebrate cadherins in its extracellular structure, the newly identified *Drosophila* cadherin, DN-cadherin [17], can still form a complex with catenins and induce cell aggregation *in vitro* [17]. DN-cadherin is expressed in all post-mitotic neurons of the CNS, and the protein is found on both axons and growth cones. Loss of DN-cadherin greatly diminishes catenin immunoreactivity in CNS axons, implying that DN-cadherin is likely to be the major cadherin in the fly CNS.

Drosophila embryos that have completely lost DN-cadherin function display only a slight distortion of the overall axon tract configuration. Abnormalities in the pattern of axon trajectories become apparent, however, when the projections of subsets of neurons are examined. These abnormalities include a failure of position shifts, defective bundling and errors in the directional migration of growth cones. Thus, DN-cadherin does not seem to be required for axonal outgrowth *per se* or for pathfinding by pioneer fibers, but rather for the proper growth and fasciculation of follower axons. Compared to the effects of DE-cadherin deficiency on epithelial structures, the disruption of the nervous system in mutant flies is relatively mild, suggesting a greater redundancy of adhesion molecules in the nervous system.

In vertebrates, the targets of cadherin-expressing neurites often express the same type cadherin, suggesting that cadherins may also be involved in target recognition or

synapse formation. In fact, recent studies have shown that, in specific regions of the brain, N-cadherin and E-cadherin are localized at synaptic clefts in a mutually exclusive fashion [21]. As two cadherin-associated proteins, αN-catenin and β-catenin, are also found in the synaptic junctions of neurons, it is thought that cadherins and catenins together form a symmetrical adhesion structure, as they do in the epithelial adherens junction, which links the presynaptic and postsynaptic membranes of the synaptic complex [22]. Compelling evidence that cadherins do have a role in synapse formation has recently been provided for N-cadherin in the retinotectal projection of the chick [23].

In the chick, retinal fibers enter the tectum through the stratum opticum, the most superficial layer of the tectum, which they then penetrate to reach deeper layers, where they arborize and form synapses. N-cadherin initially has a broad distribution, becoming concentrated in synaptic clefts only once arbors form in the three layers that receive retinal input. In a retinotectal coculture system that reproduces layer-specific neuronal outgrowth and arborization, interference with N-cadherin function has two major effects: arborization is markedly reduced, and a significant portion of axons extend beyond their normal boundaries (Figure 2). This strongly suggests that, in the tectal layers that receive retinal input, N-cadherin acts to promote or stabilize contacts between axons and their targets. Together, these findings suggest that cadherin-mediated adhesion is likely to be involved in several steps of neural circuit formation — the formation of nuclei, selective fasciculation, and finally the formation and maintenance of synapses.

Takeichi and coworkers [24] have mapped the RNA expression patterns of classical cadherins — in particular, cadherins 6, 8 and 11 — in the postnatal mouse brain. They found a distinct pattern of cadherin expression in each brain nucleus or cortical area examined. However, the neurons within a given nucleus or cortical layer do not necessarily all express the same cadherin, possibly reflecting neuronal heterogeneity. The seemingly complex patterns of cadherin expression largely appear to follow the simple rule that connected areas express the same cadherin or combination of cadherins.

The thalamocortical projections of the auditory and somatosensory systems provide particularly clear examples of this rule. The principal auditory thalamic nucleus — the medial geniculate nucleus — expresses cadherin 6, as do neurons in layer IV of the auditory (temporal) cortex, to which axons from the medial geniculate nucleus project. Similarly, cadherin 6 is also expressed in the principal thalamic sensory nucleus and its target, layer IV of parietal cortex 1 and 2. For the cerebellar system, which has topographic projections from the inferior olive to the cerebellar

cortex and to the cerebellar nuclei, significant correlations between the cadherin expression and projection patterns were also detected.

Thus, each of the classical cadherins that has been examined delineates distinct sets of neuronal circuits, but is not restricted to specific functional systems. If cadherins are indeed essential for the establishment and maintenance of neural connections, as suggested by recent functional studies, the pervasiveness and complexity of their expression in the major functional systems of the CNS implies that cadherin-mediated adhesion has a very prominent role in the formation of the highly complex neural circuitry of the brain.

References
1. Goodman CS, Shatz CJ: **Developmental mechanisms that generate precise patterns of neuronal connectivity.** *Neuron* 1993, **10**:77-98.
2. Takeichi M: **Morphogenetic roles of classic cadherins.** *Curr Opin Cell Biol* 1995, **7**:619-627.
3. Huber O, Bierkamp C, Kemler R: **Cadherins and catenins in development.** *Curr Opin Cell Biol* 1996, **8**:685-691.
4. Murphy-Erdosh C, Yoshida CK, Paradies N, Reichardt LF: **The cadherin-binding specificities of B-cadherin and LCAM.** *J Cell Biol* 1995, **129**:1379-1390.
5. Inuzuka H, Miyatani S, Takeichi M: **R-cadherin: a novel Ca²⁺-dependent cell–cell adhesion molecule expressed in the retina.** *Neuron* 1991, **7**:69-79.
6. Cepek KL, Shaw SK, Parker CM, Russel GJ, Morrow JS, Rimm DL, Brenner MB: **Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the αEβ7 integrin.** *Nature* 1994, **372**:190-193.
7. Redies C: **Cadherin expression in the developing vertebrate CNS: from neuromeres to brain nuclei and neural circuits.** *Exp Cell Res* 1995, **220**:243-256.
8. Weis WI: **Cadherin structure: a revealing zipper.** *Structure* 1995, **3**:425-427.
9. Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grübel G, Legrand J-F, Als-Nielsen J, Colman DR, Hendrickson WA: **Structural basis of cell-cell adhesion by cadherins.** *Nature* 1995, **374**:327-337.
10. Aberle H, Schwartz H, Kemler R: **Cadherin–catenin complex: protein interactions and their implications for cadherin function.** *J Cell Biochem* 1996, **61**:514-523.
11. Redies C, Takeichi M: **Cadherins in the developing central nervous system: an adhesive code for segmental and functional subdivisions.** *Dev Biol* 1996, **180**:413-423.
12. Hamaguchi M, Matsuyoshi N, Ohnishi Y, Gotoh B, Takeichi M, Nagai Y: **p60v-src causes tyrosine phosphorylation and inactivation of the N-cadherin-catenin cell adhesion system.** *EMBO J* 1993, **12**:307-314.
13. Steinberg MS, Takeichi M: **Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression.** *Proc Natl Acad Sci USA* 1994, **91**:206-209.
14. Matsunaga M, Hatta K, Nagafuchi A, Takeichi M: **Guidance of optic nerve fibres by N-cadherin adhesion molecules.** *Nature* 1988, **334**:62-64.
15. Redies C, Takeichi M: **N- and R-cadherin expression in the optic nerve of the chicken embryo.** *Glia* 1993, **8**:161-171.
16. Doherty P, Walsh FS: **Signal transduction events underlying neurite outgrowth stimulated by cell adhesion molecules.** *Curr Opin Neurobiol* 1994, **4**:49-55.
17. Iwai Y, Usui T, Hirano S, Steward R, Takeichi M, Uemura T: **Axon patterning requires DN-cadherin, a novel neuronal adhesion receptor, in the *Drosophila* embryonic CNS.** *Neuron* 1997, **19**:77-89.
18. Pai L-M, Kirkpatrick C, Blanton J, Oda H, Takeichi M, Peifer M: **Drosophila α-catenin and E-cadherin bind to distinct regions of Drosophila Armadillo.** *J Biol Chem* 1996, **271**:32411-32420.

19. Clark HF, Brentrup D, Schneitz K, Bieber A, Goodman C, Noll M: **Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in** *Drosophila*. *Genes Dev* 1995, **9**:1530-1542.

20. Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS: **The fat tumor suppressor gene in** *Drosophila* **encodes a novel member of the cadherin gene superfamily.** *Cell* 1991, **67**:853-868.

21. Fannon AM, Colman DR: **A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins.** *Neuron* 1996, **17**:423-434.

22. Uchida N, Honjo Y, Johnson KR, Wheelock MJ, Takeichi M: **The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones.** *J Cell Biol* 1996, **135**:767-779.

23. Inoue A, Sanes JR: **Lamina-specific connectivity in the brain: Regulation by N-cadherin, neurotrophins, and glycoconjugates.** *Science* 1997, **276**:1428-1431.

24. Suzuki SC, Inoue T, Kimura Y, Tanaka T, Takeichi M: **Neuronal circuits are subdivided by differential expression of type-II classic cadherins in postnatal mouse brains.** *Mol Cell Neurosci* 1997, **9**:433-448.