Cell adhesion molecules in the CNS

Toshiaki Sakisaka and Yoshimi Takai*

Department of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine/Faculty of Medicine, Suita 565-0871, Japan

*Author for correspondence (e-mail: ytakai@molbio.med.osaka-u.ac.jp)

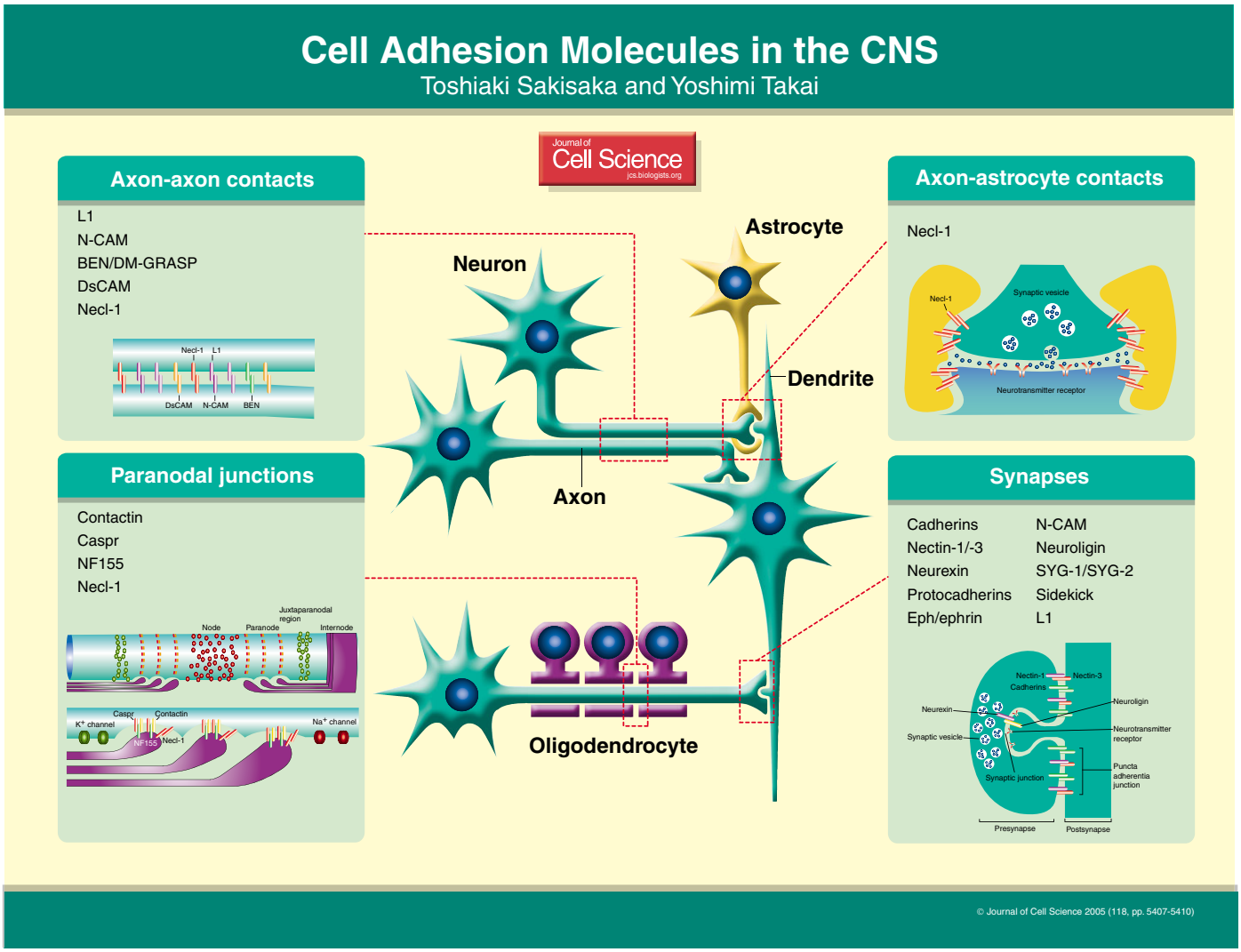
Journal of Cell Science 118, 5407-5410
Published by The Company of Biologists 2005
doi:10.1242/jcs.02672

Cell-cell adhesions are important for brain morphology and highly coordinated brain functions such as memory and learning (Sanes and Yamagata, 1999; Yamagata et al., 2003; Washbourne et al., 2004). During early development of the nervous system, neurons elongate their axons towards their targets and establish and maintain synapses through formation of cell-cell adhesions. Cell-cell adhesions also underpin axon-axon contacts and link neurons with supporting glial cells and oligodendrocytes. Here, we briefly summarize the key cell adhesion molecules involved in each case.

Synapses

Synapses are specialized intercellular junctions whose specificity and plasticity underpin the function of the nervous system. They can be subdivided into synaptic junctions (SJs) and puncta adherentia junctions (PAJs) (Peters et al., 1976). SJs are associated with presynaptic active zones, containing Ca^{2+} channels and numerous neurotransmitter-filled synaptic vesicles, and postsynaptic densities where neurotransmitter receptors localize. PAJs, by contrast, have symmetrical paramembranous dense material that lacks associated synaptic vesicles (Peters et al., 1976; Spacek and Lieberman, 1974). SJs are regarded as sites for neurotransmission, and their remodeling is thought to be a principal mechanism of memory and learning. PAJs are regarded as mechanical adhesion sites between axon terminals and their targets, although their exact functions remain unknown.

N-cadherin is a Ca^{2+}-dependent cell-cell adhesion molecule and belongs to the type I cadherin family. It has five extracellular cadherin repeat (EC) domains (EC1 to EC5) and a conserved His-Ala-Val (HAV) cell adhesion recognition sequence in the EC1



(See poster insert)

domain. Catenins are N-cadherin-binding proteins that connect N-cadherin to the actin cytoskeleton. N-cadherin and catenins symmetrically localize at PAJs between the mossy fiber terminals and the dendrites of CA3 pyramidal cells (Mizoguchi et al., 2002; Uchida et al., 1996; Takeichi, 1991; Tepass et al., 2000). In addition, the Ca^{2+}-independent immunoglobulin (Ig)-like cell-cell adhesion molecules nectin-1 and nectin-3 asymmetrically localize at the PAJ pre- and post-synaptic sides, respectively. Afadin, an actin-filament-binding protein that connects nectins to the actin cytoskeleton, is also present at PAJs (Mizoguchi et al., 2002; Takai and Nakanishi, 2003). Disruption of nectin-based cell-cell adhesion in hippocampal neurons decreases the size of synapses but increases their number (Mizoguchi et al., 2002), and a nectin-1 mutant is reported to cause human cleft lip/palate-ectodermal dysplasia, Margarita island ectodermal dysplasia and Zlotogora-Ogür syndrome, characterized by mental retardation, cleft lip/palate, syndactyly and ectodermal dysplasia (Suzuki et al., 2000). Recent work suggests cooperation of nectins and N-cadherin in the formation of synapses; however, definitive evidence has not yet been obtained.

Type II cadherins (cadherin-6, cadherin-8 and cadherin-11), which have five EC domains (EC1 to EC5) and do not have an HAV cell adhesion recognition sequence in their EC1 domains, and other type I cadherins (E- and R-cadherins) localize at the synapses with their associated catenins (Yagi and Takeichi, 2000). Multiple cadherins are differentially expressed in the brain; they could function as 'lock-and-key' components for regulating specific interneuronal connections.

Protocadherins are cell adhesion molecules that have varying numbers of EC domains but divergent cytoplasmic domains that do not appear to signal through catenins (Wu and Maniatis, 1999; Wu et al., 2001). Multiple α- and γ-protocadherin isoforms are highly expressed in distinct, although partially overlapping, sets of neurons and concentrated at synapses (Kohmura et al., 1998; Wang et al., 2002). The complex genomic organization and alternative splicing of the protocadherins has led to speculation that their diversity underlies synaptic specificity (Weiner et al., 2005).

Several Ig superfamily CAMs (for cell adhesion molecule), which have varying numbers of Ig-like domains, have been identified at synapses and shown to be involved in synaptic plasticity. For example, neural cell adhesion molecule (NCAM), a five Ig-like domain and a two fibronectin type III repeat containing protein, engages in homophilic and heterophilic interactions with a variety of ligands at synapses, such as fibroblast growth factor receptor (FGFR), L1, TAG-1/axonin-1 and heparan sulfate proteoglycans (Walsh and Doherty, 1997; Kiss and Muller, 2001). NCAM is widely expressed in the developing and adult brains and plays crucial roles in migration of neuronal precursor cells, fasciculation and pathfinding of axons, and synaptic plasticity. It is involved in both early synaptogenesis and subsequent synaptic maturation (Polo-Parada et al., 2001; Dityatev et al., 2004).

SYG-1/SYG-2 and Sidekick are specific adhesion molecules that determine synaptic specificity in a lock-and-key manner. SYG-1, a four Ig-like-domain-containing protein, and SYG-2, a seven Ig-like-domain and a fibronectin-type-III-repeat-containing protein, were isolated in a genetic screen for *C. elegans* mutants that exhibit defective synaptic positioning (Shen and Bargmann, 2003; Shen et al., 2004). SYG1-1 interacts with SYG-2 and induces formation of synapses while suppressing inappropriate synapses. Sidekick, which has six Ig-like domains and thirteen fibronectin type III repeats, has been implicated in selective synapse formation in the chicken retina (Yamagata et al., 2002).

Since the results in the synaptic localization of SynCAM1 (Biederer et al., 2002) are inconsistent with the results reported by other groups (Wakayama et al., 2001; Fukami et al., 2002; Shingai et al., 2003; Kakunaga et al., 2005), we do not describe SynCAM1 as a synaptic CAM. SynCAM1 has been shown to be ubiquitously expressed and identical to nectin-like molecule-2 (Necl-2)/IGSF4/RA175/SgIGSF/TSLC1, which is not concentrated at synapses.

Neuroligin, an esterase-like-domain-containing protein on the presynpatic side, interacts with β-neurexin, a laminin-globular-domain-containing protein on the post-synaptic side, at SJs and induces formation of synapses (Graf et al., 2004; Chih et al., 2005). However, when, where, and how precisely β-neurexin and neuroligin induce synaptogenesis remains obscure.

Eph receptor tyrosine kinases and their ephrin ligands are grouped into two families: ephrinA ligands are tethered to the plasma membrane by a glycosyl phosphatidylinositol (GPI) linkage and bind to EphA receptors; whereas ephrinB ligands are transmembrane proteins that bind preferentially to EphB receptors. EphB receptors localize to synapses, where they bind *N*-methyl-D-aspartate-type glutamate receptors. The typical Eph-ephrin interaction is not an example of classical adhesion, as described above, but rather leads to repulsion. Localized membrane shedding of ephrinB by metalloproteases converts initial adhesion to repulsion.

Axon-astrocyte contacts

Astrocytes regulate synaptic transmission by taking up glutamate from the synaptic cleft through membrane transporters and release it upon reversal of the transporter (Fields and Stevens-Graham, 2002). Other substances that astrocytes release strengthen synaptic transmission by coactivating NMDA receptors in the postsynaptic membrane (e.g. D-serine) or reduce it by binding to neurotransmitters. Synapse formation may also be regulated by factors produced by astrocytes. Thrombospondins (TSPs), for example, have been shown to play a role in formation of ultrastructurally normal synapses that are presynaptically active but postsynaptically silent (Christopherson et al., 2005).

Necl-1/TSLL1/SynCAM3, which has a domain structure similar to that of nectins, localizes at axon-astrocyte contacts (Kakunaga et al., 2005). Necl-1

shows Ca^{2+}-independent homophilic cell-cell adhesion activity and heterophilic cell-cell adhesion activity with Necl-2, nectin-1 and nectin-3, but not Necl-5 or nectin-2. It is specifically expressed in neural tissue, where it localizes to contact sites along axons, nerve terminals and glial cell processes that form synapses, axon bundles and myelinated axons.

Paranodal junctions

Oligodendrocytes, nodal glia, play an important role in the formation, organization and maintenance of myelinated axons. Three specific domains of axon-oligodendrocyte interaction are observed around the nodes of Ranvier that propagate impulses over long distances: the Na^+-channel-enriched node itself; the adjacent paranode; and the juxtaparanodal region, which contains delayed rectifier K^+ channels. This domain organization is regulated by soluble signals from myelinating glia, as well as direct contact and interactions between proteins expressed on the surface of axons and oligodendrocytes. At the paranode, the transmembrane protein Caspr is found on the axon surface in association with the GPI-anchored cell adhesion molecule contactin (Peles et al., 1997). This molecular complex interacts with the oligodendrocyte adhesion molecule neurofascin 155 (NF155) and anchors the intercellular junction to the axonal cytoskeleton through the actin-associated protein 4.1B, which binds to the cytoplasmic domain of Caspr (Charles et al., 2002; Gollan et al., 2002). Tenascin-R and tenascin-C, matrix glycoproteins, have been implicated in the formation of paranodal junctions in the CNS and PNS, respectively (Jones and Jones, 2000).

Axon-axon contacts

During early development of the nervous system, axons are guided to their targets longitudinally or through fasciculation by attractive and repulsive factors. In axon fasciculation, the best known adhesion molecules are L1 and N-CAM (Crossin and Krushel, 2000). L1 has six Ig-like domains and five fibronectin type III repeats and is a member of a large subfamily of molecules that includes Nr-CAM, Ng-CAM, neurofascin, neuroglian, aBGP and CHL1 (Moos et al., 1988). L1 interacts with other L1 molecules, integrins, N-CAM, TAG-1/axonin-1, contactin/F3/F11 and extracellular matrix molecules through its extracellular domain, and with ankyrin through its cytoplasmic domain. Studies of animals missing the molecule strongly support a role for L1 in axon guidance, and mutations in the human *L1* gene lead to a human disease characterized by mental retardation, hydrocephalus of varying severity, and defective peripheral limb movement (Fransen et al., 1997; Yamasaki et al., 1997). In part, this phenotype is due to defects in major axonal tracts, such as the corticospinal tract and the corpus callosum.

NCAM is also involved in fasciculation and pathfinding of axons. Poor axonal fasciculation is observed in the hippocampi of NCAM-deficient mice, resulting in impaired synapse formation in the CA3 region (Cremer et al., 1997). This function of NCAM appears to be mediated primarily by its polysialic acid moiety (Monnier et al., 2001).

BEN/DM-GRASP has five Ig-like domains and mediates homophilic adhesion as well as heterophilic adhesion with CD6, NgCAM and high-density lipoprotein. Poor axonal fasciculation is observed in retinal ganglion cell axons within retinal and motor axons within intercostal nerves of BEN-deficient mice, resulting in severe retinal dysplasia, including retinal folds and photoreceptor ectopias (Weiner et al., 2004).

DsCAM has ten Ig-like domains and six fibronectin type III repeats and mediates homophilic adhesion. DsCAM plays an early and essential role in promoting selective fasciculation of young axons in the peduncle of mushroom body neurons in *Drosophila* (Zhan et al., 2004).

Outlook

Given the remarkable heterogeneity of neurons and synapses in the central nervous system, it is not surprising that many cell adhesion molecules govern synapse formation and underpin other neuronal cell contacts. Functional studies of individual cell adhesion molecules have provided a wealth of information on their role in synapse assembly, spine morphogenesis and synaptic plasticity. Although the various adhesion systems can mediate adhesive interactions, individually they probably control specific aspects of synapse formation. Because multiple systems appear to cooperate at individual synapses, it will be of great interest to determine whether these act in a parallel or hierarchical manner.

We thank T. Manabe (Tokyo University, Japan), A. Mizoguchi (Mie University, Japan) and H. Togashi (RIKEN CDB, Japan) for comments on the manuscript.

References

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E. T. and Sudhof, T. C. (2002). SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science* **5586**, 1525-1531.

Charles, P., Tait, S., Faivre-Sarrailh, C., Barbin, G., Gunn-Moore, F., Denisenko-Nehrbass, N., Guennoc, A. M., Girault, J. A., Brophy, P. J. and Lubetzki, C. (2002). Neurofascin is a glial receptor for the paranodin/Caspr-contactin axonal complex at the axoglial junction. *Curr. Biol.* **12**, 217-220.

Chih, B., Engelman, H. and Scheiffele, P. (2005). Control of excitatory and inhibitory synapse formation by neuroligins. *Science* **307**, 1324-1328.

Christopherson, K. S., Ullian, E. M., Stokes, C. C., Mullowney, C. E., Hell, J. W., Agah, A., Lawler, J., Mosher, D. F., Bornstein, P. and Barres, B. A. (2005). Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* **120**, 421-433.

Cremer, H., Chazal, G., Goridis, C. and Represa, A. (1997). NCAM is essential for axonal growth and fasciculation in the hippocampus. *Mol. Cell. Neurosci.* **8**, 323-335.

Crossin, K. L. and Krushel, L. A. (2000). Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. *Dev. Dyn.* **2**, 260-279.

Dityatev, A., Dityateva, G., Sytnyk, V., Delling, M., Toni, N., Nikonenko, I., Muller, D. and Schachner, M. (2004). Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. *J. Neurosci.* **42**, 9372-9382.

Fields, R. D. and Stevens-Graham, B. (2002). New insights into neuron-glia communication. *Science* **298**, 556-562.

Fransen, E., Van, Camp, G., Vits, L. and Willems, P. J. (1997). L1-associated diseases: clinical geneticists divide, molecular geneticists unite. *Hum. Mol. Genet.* **10**, 1625-1632.

Fukami, T., Satoh, H., Fujita, E., Maruyama, T., Fukuhara, H., Kuramochi, M., Takamoto, S., Momoi, T. and Murakami, Y. (2002). Identification of the Tslc1 gene, a mouse orthologue of the human tumor suppressor TSLC1 gene. *Gene* **295**, 7-12.

Gollan, L., Sabanay, H., Poliak, S., Berglund, E. O., Ranscht, B. and Peles, E. (2002). Retention of a cell adhesion complex at the paranodal junction requires the cytoplasmic region of Caspr. *J. Cell Biol.* **157**, 1247-1256.

Graf, E. R., Zhang, X., Jin, S. X., Linhoff, M. W. and Craig, A. M. (2004). Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell* **119**, 1013-1026.

Jones, F. S. and Jones, P. L. (2000). The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. *Dev Dyn*. **218**, 235-259.

Kakunaga, S., Ikeda, W., Itoh, S., Deguchi-Tawarada, M., Ohtsuka, T., Mizoguchi, A. and Takai, Y. (2005). Nectin-like molecule-1/TSLL1/SynCAM3: a neural tissue-specific immunoglobulin-like cell-cell adhesion molecule localizing at non-junctional contact sites of presynaptic nerve terminals, axons and glia cell processes. *J. Cell Sci.* **118**, 1267-1277.

Kiss, J. Z. and Muller, D. (2001). Contribution of the neural cell adhesion molecule to neuronal and synaptic plasticity. *Rev. Neurosci.* **12**, 297-310.

Kohmura, N., Senzaki, K., Hamada, S., Kai, N., Yasuda, R., Watanabe, M., Ishii, H., Yasuda, M., Mishina, M. and Yagi, T. (1998). Diversity revealed by a novel family of cadherins expressed in neurons at a synaptic complex. *Neuron* **20**, 1137-1151.

Mizoguchi, A., Nakanishi, H., Kimura, K., Matsubara, K., Ozaki-Kuroda, K., Katata, T., Honda, T., Kiyohara, Y., Heo, K., Higashi, M. et al. (2002). Nectin: an adhesion molecule involved in formation of synapses. *J. Cell Biol.* **3**, 555-565.

Monnier, P. P., Beck, S. G., Bolz, J. and Henke-Fahle, S. (2001). The polysialic acid moiety of the neural cell adhesion molecule is involved in intraretinal guidance of retinal ganglion cell axons. *Dev Biol.* **229**, 1-14.

Moos, M., Tacke, R., Scherer, H., Teplow, D., Fruh, K. and Schachner, M. (1988). Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature* **6184**, 701-703.

Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G. D. and Schlessinger, J. (1997). Identification of a novel contactin-associated transmembrane receptor with multiple domains implicated in protein-protein interactions. *EMBO J.* **16**, 978-988.

Peters, A., Palay, S. L. and Webster, H. (1976). *The Fine Structure of the Nervous System: The Neurons and Their Supporting Cells.* Oxford University Press, New York.

Polo-Parada, L., Bose, C. M. and Landmesser L. T. (2001). Alterations in transmission, vesicle dynamics, and transmitter release machinery at NCAM-deficient neuromuscular junctions. *Neuron* **32**, 815-828.

Sanes, J. R. and Yamagata, M. (1999). Formation of lamina-specific synaptic connections. *Curr. Opin. Neurobiol.* **9**, 79-87.

Shen, K. and Bargmann, C. I. (2003). The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. elegans. *Cell* **112**, 619-630.

Shen, K., Fetter, R. D. and Bargmann, C. I. (2004). Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. *Cell* **116**, 869-881.

Shingai, T., Ikeda, W., Kakunaga, S., Morimoto, K., Takekuni, K., Itoh, S., Satoh, K., Takeuchi, M., Imai, T., Monden, M. and Takai, Y. (2003). Implications of nectin-like molecule-2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1 in cell-cell adhesion and transmembrane protein localization in epithelial cells. *J. Biol. Chem.* **278**, 35421-35427.

Spacek, J. and Lieberman, A. R. (1974). Three dimensional reconstruction in electron microscopy of the central nervous system. *Sb. Ved. Pr. Lek. Fak. Karlovy Univerzity Hradci. Kralove.* **3**, 203-222.

Suzuki, K., Hu, D., Bustos, T., Zlotogora, J., Richieri-Costa, A., Helms, J. A. and Spritz, R. A. (2000). Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. *Nat. Genet.* **4**, 427-430.

Takai, Y. and Nakanishi, H. (2003). Nectin and afadin: novel organizers of intercellular junctions. *J. Cell Sci.* **1**, 17-27.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **5000**, 1451-1455.

Tepass, U., Truong, K., Godt, D., Ikura, M. and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. *Nat. Rev. Mol. Cell. Biol.* **2**, 91-100.

Uchida, N., Honjo, Y., Johnson, K. R., Wheelock, M. J. and Takeichi, M. (1996). The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones. *J. Cell Biol.* **3**, 767-779.

Wakayama, T., Ohashi, K., Mizuno, K. and Iseki, S. (2001). Cloning and characterization of a novel mouse immunoglobulin superfamily gene expressed in early spermatogenic cells. *Mol. Reprod. Dev.* **60**, 158-164.

Walsh, F. S. and Doherty, P. (1997). Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. *Annu. Rev. Cell. Dev. Biol.* **13**, 425-456.

Wang, X., Weiner, J. A., Levi, S., Craig, A. M., Bradley, A. and Sanes, J. R. (2002). Gamma protocadherins are required for survival of spinal interneurons. *Neuron.* **36**, 843-854.

Washbourne, P., Dityatev, A., Scheiffele, P., Biederer, T., Weiner, J. A., Christopherson, K. S. and El-Husseini, A. (2004). Cell adhesion molecules in synapse formation. *J. Neurosci.* **42**, 9244-9249.

Weiner, J. A., Koo, S. J., Nicolas, S., Fraboulet, S., Pfaff, S. L., Pourquie, O. and Sanes, J. R. (2004). Axon fasciculation defects and retinal dysplasias in mice lacking the immunoglobulin superfamily adhesion molecule BEN/ALCAM/SC1. *Mol. Cell Neurosci*. **27**, 59-69.

Weiner, J. A., Wang, X., Tapia, J. C. and Sanes, J. R. (2005). Gamma protocadherins are required for synaptic development in the spinal cord. *Proc. Natl. Acad. Sci. USA* **102**, 8-14.

Wu, Q. and Maniatis, T. (1999). A striking organization of a large family of human neural cadherin-like cell adhesion genes. *Cell* **6**, 779-790.

Wu, Q., Zhang, T., Cheng, J. F., Kim, Y., Grimwood, J., Schmutz, J., Dickson, M., Noonan, J. P., Zhang, M. Q., Myers, R. M. et al. (2001). Comparative DNA sequence analysis of mouse and human protocadherin gene clusters. *Genome Res.* **3**, 389-404.

Yagi, T. and Takeichi, M. (2000). Cadherin superfamily genes: functions, genomic organization, and neurologic diversity. *Genes Dev.* **14**, 1169-1180.

Yamagata, M., Weiner, J. A. and Sanes, J. R. (2002). Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. *Cell* **110**, 649-660.

Yamagata, M., Sanes, J. R. and Weiner, J. A. (2003). Synaptic adhesion molecules. *Curr. Opin. Cell Biol.* **5**, 621-632.

Yamasaki, M., Thompson, P. and Lemmon, V. (1997). CRASH syndrome: mutations in L1CAM correlate with severity of the disease. *Neuropediatrics* **3**, 175-178.

Zhan, X. L., Clemens, J. C., Neves, G., Hattori, D., Flanagan, J. J., Hummel, T., Vasconcelos, M. L., Chess, A. and Zipursky, S. L. (2004). Analysis of Dscam diversity in regulating axon guidance in Drosophila mushroom bodies. *Neuron* **43**, 673-686.

Cell Science at a Glance on the Web

Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.