CHAPTER

9

Cell Adhesion Molecules

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OUTLINE

OVERVIEW

Cell adhesion molecules are cell-surface proteins that account for cell-to-cell and/or cell-to-extracellular matrix (ECM) interactions. Cell adhesion molecules act at the cell surface, where they interact with molecules that are expressed on an opposing cell surface or present in the ECM. Thus they engage in *trans* interactions with either identical molecules, termed homophilic interactions, or with different molecules, termed heterophilic interactions. In the nervous system, cell adhesion molecules have been shown to play critical roles in all facets of nervous system development and maintenance, and many cell adhesive mechanisms between many different types of cells have been described (Sakisaka & Takai, 2005; Shapiro et al., 2007). As an example, the establishment and maintenance of precise synaptic connections between neurons is regulated by distinct adhesive mechanisms and is crucial for the proper flow of information within neural networks. It is important to note that while cell adhesion molecules have often been identified via their adhesive 'sticky' properties, their adhesive interactions are associated with changes in intracellular signaling, cytoskeletal organization and/or gene expression. Thus, the phenomenon of cell adhesion represents an active cellular process. Interestingly, the biological effects that result from interactions mediated by cell adhesion molecules can be either adhesive or repulsive (inhibitory) in

nature. This concept is probably best illustrated by the existence of attractive as well as repulsive cues guiding axonal and dendritic growth cones during development (Tessier-Lavigne & Goodman, 1996). However, there are always many adhesion molecules expressed at the same time on a given cell surface, and intercellular adhesion may be modulated by other signaling systems. Thus, the decision on whether the response to cell adhesion is adhesion (attraction) or repulsion (inhibition) depends in most cases on the temporally and spatially controlled net result of all cell adhesion molecule interactions occurring at the cell surface.

Cell adhesion molecules comprise several 'superfamilies'

Cell adhesion molecules are divided into four major groups or 'superfamilies': the immunoglobulin superfamily of cell adhesion molecules (IgCAMs, Figs. 9-1 to 9-4), the cadherins (Figs. 9-5, 9-6), the integrins (Figs. 9-7, 9-8) and the superfamily of C-type lectin-like domain proteins (CTLDs). Of these, IgCAMs, cadherins and integrins have been functionally implicated in a number of processes in the nervous system, while CTLDs have been primarily characterized in the immune system. CTLDs contain the so-called carbohydrate-recognition domain (CRD) of calcium-dependent (C-type) carbohydrate binding proteins (lectins) or a domain highly homologous to it (Zelensky & Gready, 2005). They include the subfamily of selectins, which are functionally involved in the recruitment of lymphocytes into the inflamed CNS. Likewise, lecticans, extracellular proteoglycans representing an important component of the CNS ECM, are considered part of the CTLD superfamily (Yamaguchi, 2000). In addition to the above, the netrin/neurexin and semaphorin families of cell adhesion molecules have been described in the nervous system, and have recently gained increased attention due to their functional roles in synapse formation and synaptic plasticity. Netrins, neuroexins and their neuroligin receptors are one of the best-known cell adhesion molecule systems involved in regulating synaptic differentiation (Fig. 9-10). Semaphorins and their plexin and neuropilin co-receptors were originally discovered as evolutionarily conserved molecules regulating the guidance of axonal growth cones and have more recently been implicated in the regulation of synapse formation and synaptic plasticity (Pasterkamp & Giger, 2009).

IMMUNOGLOBULIN SUPERFAMILY

The immunoglobulin (Ig)-like domain is a typical feature of proteins belonging to the immunoglobulin superfamily

The immunoglobulin (Ig)-like domain is a protein domain that is similar in amino acid sequence and structure to the Ig domains of immunoglobulins (Edelman, 1987; Williams & Barclay, 1988). Structurally, Ig domains possess a distinctive immunoglobulin fold composed of 70–110 amino acids (Fig. 9-1). The immunoglobulin fold is defined by two opposing antiparallel β-sheets that are connected in a unique manner. The core of the immunoglobulin fold is formed by the four β-strands (B, C, E, and F) of one sheet embedded in an antiparallel curled β-sandwich with the three to five β-strands (A, C′, C″, D, and G) of the other sheet (Bork et al., 1994). A central disulfide bond is universally found to span the domain core. Of the different topological subtypes of Ig-like domains, the most common ones are the immunoglobulin constant (C-) and variable (V-) type domains, which are named according to their occurrence in the constant and variable regions of immunoglobulins, respectively (Fig. 9-1). The C-type domains are further subdivided into C1- and C2- (or s) type domains (Smith & Xue, 1997). C1-type domains are found in immunoglobulins, T-cell receptors and major histocompatibility complex (MHC) proteins, while C2-type domains are present in non-immunoglobulin-related molecules. In addition, an I- (or h)-type domain with an intermediate structure between the V- and C-type domains is frequently present in cell-surface receptors. Ig-like domains rarely occur in isolation but typically form concatenated chains, often with a V- or I-type domain at the N-terminus.

Ig-like domains are frequently found protein domains. For example, in the human genome, there are more than 750 identified proteins with at least one Ig-like domain. Prominent examples are, in addition to the above-mentioned immunoglobulin-related molecules, receptor tyrosine kinases, co-receptors such as CD4 or CD8 and many cell adhesion molecules. Evolutionarily, development of the immunoglobulin superfamily is associated with mutations, duplications and translocations of the genes encoding both V- and C-type Ig-like domains, thus yielding proteins with different numbers and combinations of these domains.

Cell adhesion molecules of the immunoglobulin superfamily (IgCAMs) represent a diverse group of proteins

IgCAMs belong to one of the most ancient families of cell adhesion molecules. Thus, most vertebrate IgCAMs have invertebrate orthologs. IgCAMs are expressed by various cell types of almost all organs. They predominantly mediate calcium-independent interactions between cells that can be either homophilic (IgCAM A binds to IgCAM A) or heterophilic (IgCAM A binds to IgCAM B). However, IgCAMs can also be involved in heterophilic interactions between cells and the ECM. The functions of IgCAMs are best characterized in the immune system and in the nervous system (Maness & Schachner, 2007; Murphy et al., 2007). Like all other cell adhesion molecules and in common with all secreted and extracellular orientated proteins, IgCAMs are glycosylated in a cell type–specific manner. In some cases, nearly half of the apparent molecular weight is represented by glycans. Moreover, nearly any IgCAM may have isoforms that differ in the size of the intracellular domains or the presence of various extracellular domains. The presence of different IgCAM isoforms can have various consequences. For example, one isoform may be a transmembrane protein while the other may be anchored to the plasma membrane via glycosylphosphatidylinositol (GPI).

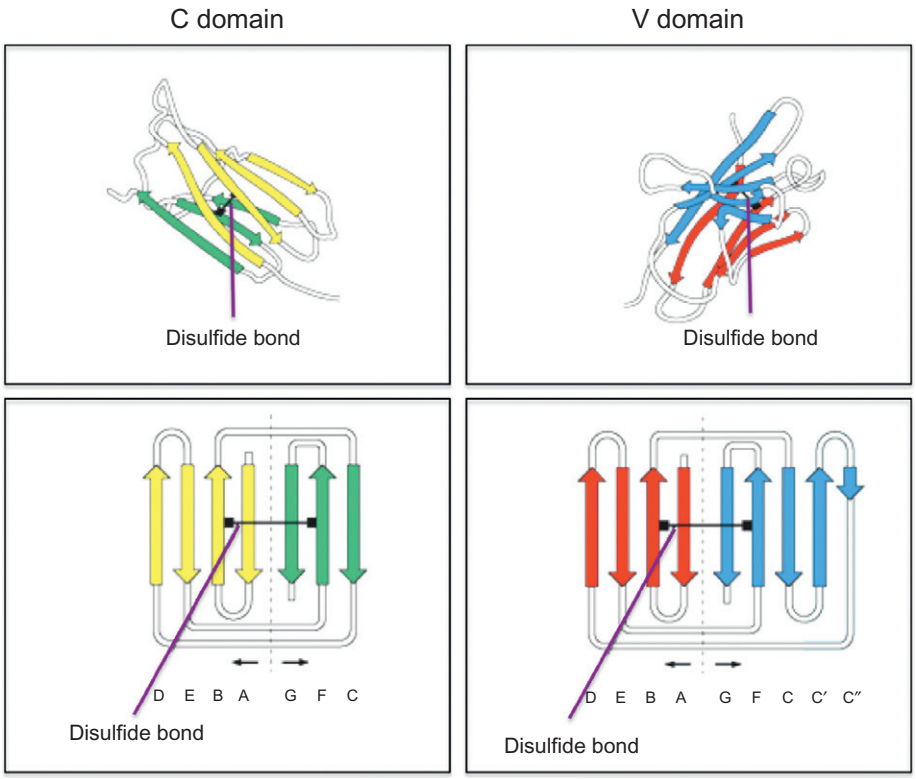

FIGURE 9-1 **Structure of immunoglobulin constant (C) and variable (V) domains—the immunoglobulin fold.** Each immunoglobulin domain contains 7 to 9 antiparallel polypeptide chains, so-called β-strands (yellow and green in the diagram of the C domain; blue and red in the diagram of the V chain). These β-strands are arranged in two β-sheets to form a β-sandwich structure that is held together by a disulfide bond. As shown in the 'opened' diagrams at the bottom, the β-strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence. The β-strands C′ and C″ are found in V domains but not in C domains. These characteristic four-strand-plus-three-strand (C-type domain) or four-strand-plus-five-strand (V-type domain) arrangements are typical immunoglobulin superfamily domain building blocks. Their distinctive folded structure is referred to as the immunoglobulin fold. (Adapted from Murphy et al., 2007).

IgCAMs can be subdivided into two major subgroups: proteins with one or more Ig-like domain(s) and proteins with Ig-like domains and additional fibronectin type III repeats (Fig. 9-2). Examples of IgCAMs with only one Ig domain are P_0 and Thy-1. P_0 is in terrestrial vertebrates the most abundant protein present in peripheral nervous system (PNS) myelin, a multi-lamellar membrane that surrounds axonal segments and allows fast and efficient propagation of electrical signals by a mechanism that is referred to as saltatory conduction. P_0 is thought to mediate, in part via its Ig-like domains, homophilic adhesive interactions between the membranes of the multi-lamellar myelin sheath and to thus represent in terrestrial vertebrates a key structural constituent of PNS myelin (Shapiro et al., 2007). Thy-1 expression in the nervous system is predominantly neuronal but some glial cells also express Thy-1, especially at later stages of their differentiation. In general, Thy-1 levels in the neonatal and developing brain are low compared to levels found in the adult brain. It is currently thought that continued Thy-1 expression in mature neurons might play a role in stabilizing them and their synaptic junctions (Bradley et al., 2009).

A well-known example of an IgCAM with more than one Ig-like domain is the myelin-associated glycoprotein (MAG). MAG is a myelin component that is present in both the central nervous system (CNS) and the PNS, and it is functionally involved in myelination during development and regeneration. It is notable that MAG is the only neural member of a subset of sialic-acid–binding IgCAM lectins, the siglecs (Crocker et al., 2007). Siglecs are thought to promote cell–cell interactions and to regulate the functions of cells, mainly in the innate and adaptive immune systems, through sialylated glycan recognition. There are currently 13 human and 9 murine known siglecs. According to the common nomenclature, MAG is referred to as siglec-4.

Prominent examples for IgCAMs with Ig domains and additional fibronectin type III repeats are the neural cell adhesion molecule (NCAM) and L1 (Maness & Schachner, 2007). NCAM is the prototype IgCAM. Already described in the early 1970s, NCAM is the 'oldest' neural cell adhesion molecule. However, NCAM is unique in that it bears an unusual glycan structure in form of long polymers of a sialic acid referred to as polysialic acid (Hildebrandt et al., 2010 and see Fig. 9-3). This glycan structure is rarely found on any other glycoprotein and has distinct functions in regulating neural development and synaptic plasticity. Furthermore, NCAM is expressed in three major isoforms that

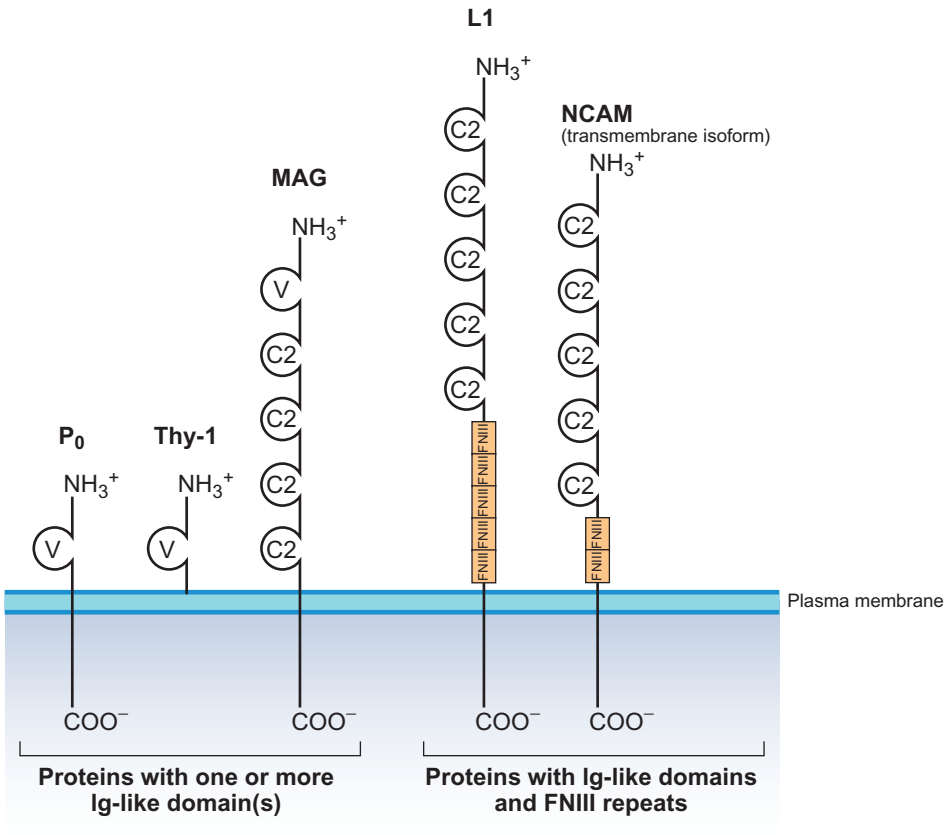

FIGURE 9-2 **Cell adhesion molecules of the immunoglobulin superfamily (IgCAMs).** IgCAMs can be subdivided into two major subgroups: proteins with one or more Ig-like domain(s), and proteins with Ig-like domains and additional fibronectin type III repeats. V, V-type Ig-like domain; C2, C2-type Ig-like domain; MAG, myelin-associated glycoprotein; NCAM, neural cell adhesion molecule; FNIII, fibronectin type III.

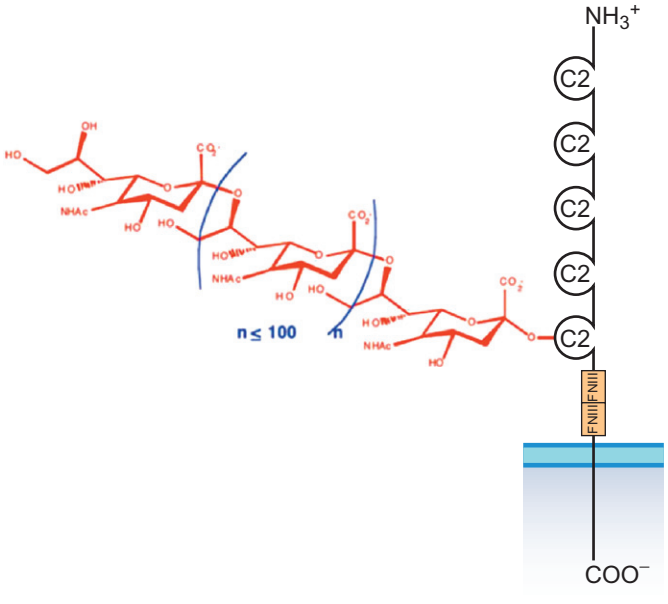

FIGURE 9-3 **Polysialylated neural cell adhesion molecule (PSA-NCAM).** The addition of up to 100 sialic acid residues to a complex N-glycan located at the fifth Ig-like domain is a unique feature of NCAM.

are generated by alternative splicing from one single gene. With reference to their apparent molecular weights, they are termed NCAM 120, NCAM 140 and NCAM 180. NCAM 120 is anchored to the membrane via glycosylphosphatidylinositol (GPI), whereas NCAM 140 and NCAM 180 are transmembrane glycoproteins with large intracellular domains of different lengths. NCAM expression is not restricted to the nervous system. It is, for example, also expressed in the immune system, where it is referred to as CD56.

The ectodomain structure of IgCAMs, and specifically the presence of Ig-like domains and additional fibronectin type III repeats, is shared by the receptors of the Roundabout (Robo) family, which have well-described roles in axon guidance by interacting with soluble Slit ligands (Ypsilanti et al., 2010). In addition, Robos can mediate homophilic interactions. The standard Robo receptor contains five Ig-like domains, three fibronectin type III repeats and a large, mainly unstructured cytosolic domain.

IgCAMs signal to the cytoplasm

Proteins belonging to the immunoglobulin superfamily are capable of initiating signal transduction events via their cytoplasmic domains and upon an adhesive interaction.

Classical examples are growth factor receptors, such as Trk receptors for nerve growth factor (NGF) and brain-derived nerve growth factor (BDNF), or the fibroblast growth factor (FGF) receptor. All these receptors have in common the presence of extracellular Ig-like domains and intracellular tyrosine kinase domains that are activated after ligand binding.

IgCAMs such as NCAM and L1 can also act as signal transducers, however, without having an intracellular domain with enzymatic activity, as illustrated for the signal transduction cascades triggered by NCAM (Fig. 9-4). There are two principal pathways for signal transduction via NCAM (Ditlevsen & Kolkova, 2010; Maness & Schachner, 2007). First, homophilic *trans* interaction between NCAM molecules leads to binding of the non-receptor tyrosine kinase p59fyn to the intracellular domain of NCAM and subsequent activation of focal adhesion kinase (FAK) and the Ras-Raf-MAPK (MEK) pathway. Second, heterophilic *trans* interaction between NCAM and the FGF receptor leads to an increase in intracellular calcium and the activation of calcium/calmodulin–dependent protein kinase II (CaMKII). Both pathways ultimately lead to the activation of the transcription factor cyclic AMP response element–binding protein (CREB). This activation of CREB and the transcription of CREB-activated genes are required for NCAM-stimulated neurite outgrowth. In addition, NCAM-mediated cell–cell interactions and their roles in processes related to synaptic plasticity, including learning and memory formation, have been associated with the activation of CREB. These findings highlight the functional importance of intracellular signal transduction events that are initialized by adhesive interactions mediated by IgCAMs.

CADHERINS

The extracellular cadherin (EC) repeat is a typical feature of cadherins

The cadherins are a superfamily of transmembrane proteins that have been classified into several subfamilies based on sequence comparisons and protein structural data (Fig. 9-5 and Hulpiau & van Roy, 2009; Nollet et al., 2000). Common to all cadherins is the presence of at least two extracellular cadherin (EC) repeats. Each EC repeat of approximately 110 residues folds into a structural unit comprising seven β strands that form two β sheets, a topology similar to the Ig-like domain. The EC structural units are rigidified by binding of calcium ions, and they provide intermolecular surfaces mediating adhesion and cell–cell contact. The necessity of calcium for adhesive function is the basis for the name cadherin, an approximate contraction of 'calcium-dependent adherent protein.'

Full functionality of cadherins requires in addition to adhesion a structural linkage with the cytoskeleton. This linkage is mediated by specific protein complexes that are bound to the cytoplasmic parts of cadherins. For example, type I and II cadherins are linked in adherens junctions to the actin cytoskeleton through catenin proteins, while desmosomal cadherins are connected in desmosomal junctions to intermediate filaments through plakoglobin (γ-catenin), desmoplakin and plakophilin proteins. The biological functions of cadherins are diverse and include the regulation of cell recognition (Geiger & Ayalon, 1992; Takeichi, 1991); tissue morphogenesis (Gumbiner, 2005); tumor progression (Blaschuk & Devemy, 2009; Jeanes et al., 2008); synapse formation and synaptic activity (Suzuki & Takeichi, 2008; Takeichi, 2007).

The type I ('classic') cadherins are homophilic cell adhesion molecules

The best-studied subfamily of the cadherins is the type I subfamily, which has been shown to function in *trans* homophilic adhesion. That is, cells expressing a particular cadherin will preferentially adhere to cells expressing the same cadherin. It was originally thought that all cadherins would behave completely homophilically, but it is now clear that, for example, N-cadherin can bind to R-cadherin, although perhaps more weakly than it would to N-cadherin. Crystal structure data, such as those obtained for C-cadherin (PE-cadherin), postulate that calcium-bound type I cadherins adopt a stable curved structure over the length of their extracellular domains (Fig. 9-6 and Boggon et al., 2002; Pokutta & Weis, 2007). In this structure, the boundaries between the EC repeats are made rigid by three ligated calcium ions and the adhesive binding site is localized primarily within the EC1 repeat, the N-terminal and membrane distal EC repeat. The so-called 'strand dimer' or *trans* interface between EC1 repeats of two opposing cadherins present on different cells is formed by an exchange of N-terminal β-strands between the two EC1 repeats and by insertion of a conserved Trp2 side chain from one EC1 repeat into the hydrophobic core of the other EC1 repeat (Fig. 9-6). The 'strand dimer' interface is thought to be the major determinant of cadherin specificity. However, little is currently known about the exact nature of the residues and factors that determine the binding preferences of cadherins. In addition to the *trans* interface, a *cis* interface between cadherin molecules present on the same cell may be formed by the interaction of the EC1 repeat of one molecule with the EC2 repeat of the neighboring molecule. The combination of *cis* and *trans* interactions predicts the formation of a protein lattice, and not, as previously believed, an adhesion zipper. Studies using truncated cadherin proteins further suggest that the EC repeats following EC2 contribute to the homophilic interaction without directly mediating adhesion.

In contrast to the relatively well-characterized molecular mechanism of *trans* homophilic adhesion between type I cadherins, much less is known about the structure–function relationships for other cadherin subfamilies. It appears, however, that there are substantial differences between the subfamilies. For example, protocadherins are characterized by EC repeats that lack strong *trans* homophilic adhesion activity (Morishita & Yagi, 2007). Such differences likely preclude adhesive interactions between members of different subfamilies.

Cadherins are involved in multiple processes in the nervous system

At least three to four dozen cadherins from different subfamilies are expressed in the nervous system (Redies, 2000). Most of these are present relatively early during development. For example, the type I cadherin N-cadherin is expressed by the proliferative neuroepithelium, where it is functionally important for maintaining adherens junctions and thereby holding

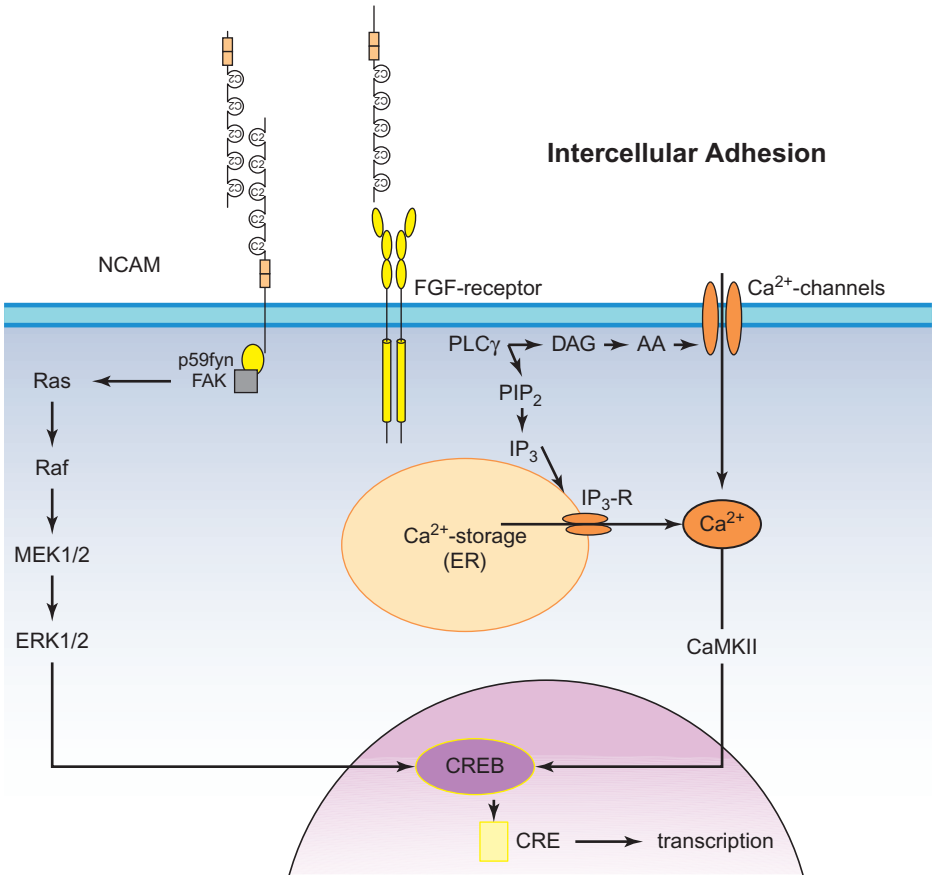

FIGURE 9-4 **NCAM-triggered signaling pathways.** Two NCAM-triggered signaling pathways lead to the phosphorylation and activation of the cyclic AMP response element (CRE)-binding transcription factor CREB. In the first one (left side of the diagram), homophilic *trans* interaction between NCAM molecules activates the non-receptor tyrosine kinase p59fyn initiating recruitment and activation of focal adhesion kinase (FAK). Subsequent signaling through the Ras-Raf-MEK1/2-ERK1/2 pathway leads to the activation of CREB. In the second pathway (right side of the diagram), NCAM binding to the fibroblast growth factor (FGF) receptor induces phospholipase Cγ (PLCγ)-mediated generation of diacylglycerol (DAG) and arachidonic acid (AA), leading to the activation of calcium channels. In addition, activation of PLCγ generates inositol trisphosphate (IP3), which is known to stimulate release of calcium from the endoplasmic reticulum (ER). Subsequent increase in intracellular calcium levels is thought to activate calcium/calmodulin-dependent protein kinase II (CaMKII) and CREB.

neuroepithelial or radial glial cells together at the ventricular side of the developing cortex. With the exception of this relatively ubiquitous expression of N-cadherin in the early developing neuroepithelium, cadherin expression is rather selective and restricted to subregions, neuronal subpopulations and even fiber tracts. Most interestingly, fiber tracts expressing a certain cadherin often connect gray matter structures expressing the same cadherin. Thus, an entire neural circuit can be selectively marked by the expression of a particular cadherin. It is of note that the clustered protocadherins, the largest and most diverse cadherin subfamily with over 50 members derived from three gene loci, are expressed predominantly in the brain (Yagi, 2008). More specifically, they are found in neurons and often localized to synapses. One of its members, Pcdh-α, was therefore originally referred to as cadherin-related neuronal receptor (CNR). While the precise functions of clustered protocadherins have yet to be elucidated, initial genetic studies in mice suggest that the expression of different members of this cadherin subfamily might specify both the survival and the synaptic organization of different neuronal populations.

INTEGRINS

Integrins are the major cell surface receptors responsible for cell adhesion to extracellular matrix (ECM) proteins

In vertebrates integrins have also been shown to play important roles in specialized cell–cell adhesive mechanisms by interacting with proteins of the immunoglobulin superfamily (Hynes, 2002). Integrins are transmembrane heterodimers comprised of non-covalently linked α and β subunits, each with a large extracellular domain, a single transmembrane domain and a generally short cytoplasmic tail. In mammals, there are 18 α and 8 β subunits, which assemble into 24 known integrins with different ligand specificities (Fig. 9-7). Both integrin subunits contribute to the formation of the ligand-binding site, the so-called ligand binding αβ headpiece, which for most integrins recognizes relatively short peptide sequences within large protein ligands. A well-characterized motif present in a number of integrin ligands is the amino acid

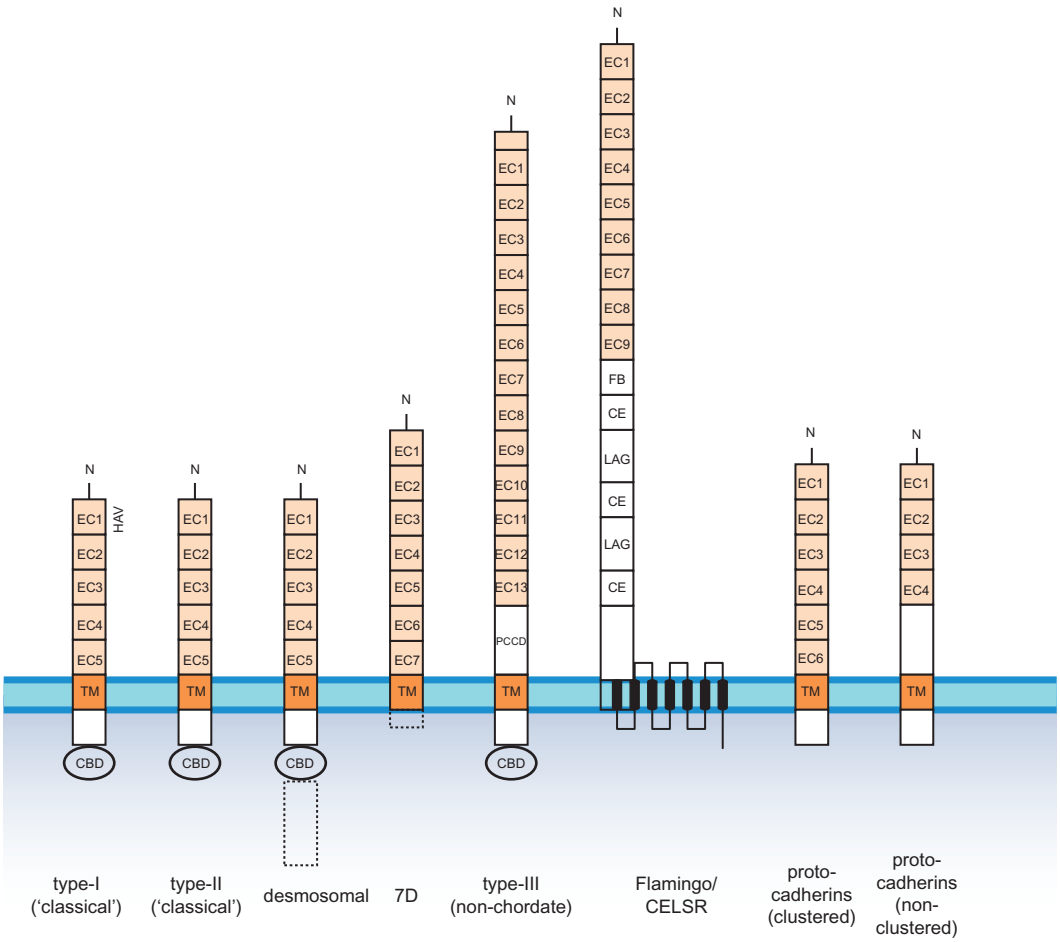

FIGURE 9-5 **Main cadherin subfamilies.** Type I, type II and desmosomal cadherins are characterized by the presence of five extracellular cadherin repeats (EC) and an intracellular catenin-binding domain (CBD). Only type I cadherins contain a conserved His-Ala-Val (HAV) cell adhesion recognition sequence in their first EC domain. Desmosomal cadherins contain in part an extended cytoplasmic domain (dotted box), and their CBD differs from type I and type II cadherins in its predominant binding to plakoglobin (γ-catenin). 7D cadherins have seven EC repeats and have so far not been described to be expressed in the nervous system. Type III cadherins have 13 EC repeats followed by a primitive classic cadherin domain (PCCD) that contains the so-called non-chordate motif, cysteine-rich EGF repeat-like motifs and laminin globular domain-like motifs. Type III cadherins exist in invertebrates and restricted vertebrate species but not in mammals. Flamingo/CELSR (cadherin EGF LAG seven-pass G-type receptor) cadherins have nine EC repeats followed by alternating cysteine-rich EGF repeat-like (CE) and laminin globular domain-like (LAG) motifs. All Flamingo/CELSR cadherins are seven-pass transmembrane proteins, which is a unique feature within the cadherin superfamily. Protocadherins are characterized by EC repeats that lack strong trans homophilic adhesion activity. In addition, protocadherins' cytoplasmic regions do not have catenin binding domains. Protocadherins are divided in two groups: clustered protocadherins that genomically are found clustered within a genome locus, and nonclustered protocadherins that do not have such a specific clustered genome locus. Clustered protocadherins have six EC repeats, while nonclustered protocadherins can have various numbers of EC repeats. Not shown here is cadherin 13 (T-or H-cadherin), which, similar to type II cadherins, has five EC repeats with strong adhesive properties but is the only known cadherin to be attached to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. In addition, not depicted are cadherins of the Dachsous/Fat subfamily, which are characterized by arrays of up to 34 EC repeats, making them the largest cadherin molecules.

sequence Arg-Gly-Asp (RGD), which was originally identified as the minimal integrin recognition sequence present in the prototypic integrin ligand fibronectin (Ruoslahti, 1996). At least eight of the currently known integrins recognize RGD-containing ligands, and the RGD sequence is thus often considered a general integrin-binding motif. However, not all of the integrins have been found to bind to the RGD sequence (Fig. 9-7). In addition, availability and contextual presentation of the RGD sequence are important for integrin binding. As a consequence, not all molecules containing the RGD sequence are recognized by RGD-binding integrins. For example, RGD sequences present in triple helical fibrillar collagen sequences are normally not available at the surface and are thus not recognized by integrins. Furthermore, there is additional specificity in integrin binding to RGD-containing ligands in that each of the RGD-binding integrins recognizes only a specific ligand or specific set of ligands. For example, the integrin $\alpha v\beta 3$ recognizes vitronectin and fibrinogen in addition to fibronectin, while one of the major fibronectin receptors, the integrin $\alpha 5\beta 1$, does not recognize either of the above additional ligands. This

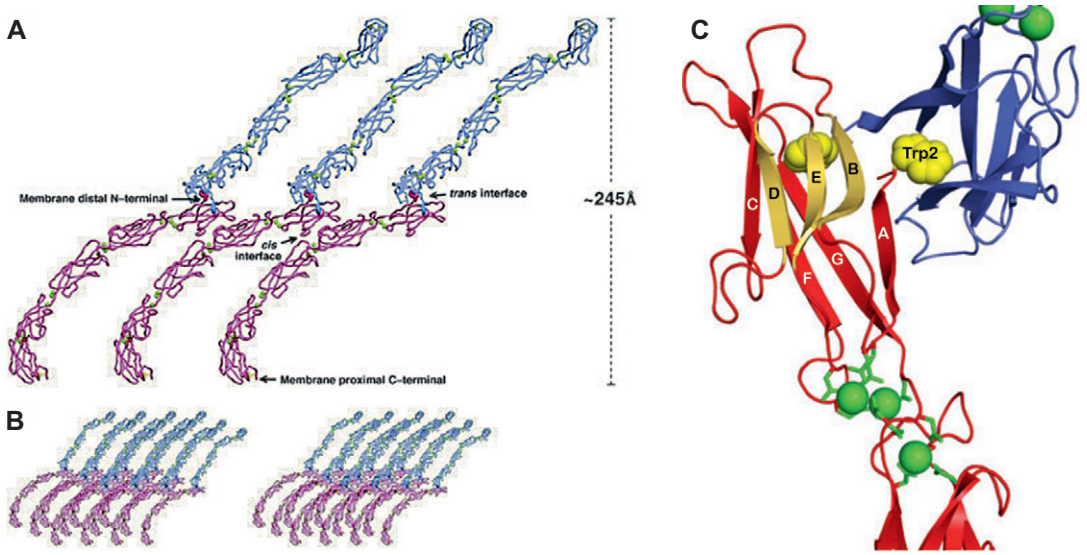

FIGURE 9-6 **Structure of cadherins and extracellular cadherin (EC) repeats. (A)** The extracellular domains of the type I cadherin C-cadherin (EP-cadherin) are oriented as if protruding from opposing cell surfaces. Note that *trans* interfaces, also referred to as cadherin 'strand dimers,' are mediated through interactions between the EC1 repeats of molecules emanating from opposing cell surfaces, and that calcium ions (green dots) are ligated at the interfaces between successive EC repeats. The *cis* and *trans* interfaces together determine the formation of a protein lattice. **(B)** Stereo view of the three-dimensional cadherin lattice. (A and B from Boggon et al., 2002). **(C)** EC repeats form a seven-stranded β-sandwich structure. β-strands are labeled A–G; strands C, F, G, and A (*red*) form one sheet, and strands D, E, and B (yellow) form the other sheet of the β-sandwich. Three calcium ions (green) are ligated between successive EC repeats. At the *trans* interface, the conserved Trp2 side chain (yellow) from one EC1 repeat (red) is inserted into the hydrophobic core of the other EC1 repeat (blue), and vice versa. (C adapted from Pokutta & Weis, 2007).

example also illustrates that in contrast to early assumptions, most integrins are not specific for single ligands, and many of the integrin-binding ligands are recognized by multiple integrins. At first glance, it therefore appears that there may be significant redundancy in the function of integrins. However, the generation and analysis of knockout mice lacking different integrin subunits reveals distinct phenotypes, thus suggesting the existence of specific, nonredundant functions for each of the integrins (Hynes, 2002).

Integrin function is characterized by the formation of a linkage between the cytoskeleton of the integrin-expressing cell and its extracellular ligand (Delon & Brown, 2007; Zaidel-Bar & Geiger, 2010). This functional importance is reflected in the name integrin, which refers to 'maintaining the integrity of the cytoskeletal-ECM linkage.' For almost all integrins this linkage is to the actin cytoskeleton. There is currently no evidence to suggest that integrins bind actin directly. Instead they recruit a large multi-molecular complex of proteins that then indirectly provides the linkage to the actin cytoskeleton. The interactions between the more than 150 proteins thought to be involved in this integrin–actin connection are often transient, and it is this dynamic nature that makes this connection particularly sensitive and responsive to changes in the extracellular environment. The integrin α6β4 is the only integrin known to not connect to the actin cytoskeleton but to associate instead, via its β4 subunit, to intermediate filaments.

The interactions between integrins and their ligands play important roles in most biological and cellular processes. In the nervous system the roles of integrins are probably best characterized in the PNS, where they are critical for the formation of the neuromuscular junction, the synaptic connection between the terminal of a motor neuron and a muscle fiber, and the development of glial cells, namely Schwann cells (Berti et al., 2006; Hortsch & Umemori, 2009). In the CNS integrins are expressed by a variety of cell types including neurons, glial and endothelial cells. The proposed functions of integrins in the CNS cover a large range of processes during normal development and in the adult (Clegg et al., 2003). These include cell migration, differentiation, axon and dendrite outgrowth, synapse formation, synaptic plasticity and myelination. In addition, integrins mediate lymphocyte entry into the inflamed CNS (see Box 9-1), and they play important roles in modulating the behavior of microglia, the resident immune cells of the CNS, following injury or infection (Milner & Campbell, 2002).

Integrins signal in an inside-out and outside-in fashion

Integrins are somewhat unusual among transmembrane receptors in that they signal bidirectionally (Fig. 9-8 and Hynes, 2002; Shattil et al., 2010). First, integrin function can be regulated from within a cell, a concept that is referred to as inside-out signaling. Second, binding of extracellular ligands to integrins can activate intracellular signaling pathways and thus affect cellular function via outside-in signaling.

Regulation of integrin function via inside-out signaling is particularly important under conditions where integrins are constantly exposed to their ligands. For example, the platelet integrin αIIbβ3 is inactive on resting platelets but rapidly activated in response to injury to bind to its ligand fibrinogen and to stimulate platelet aggregation. The tight regulation

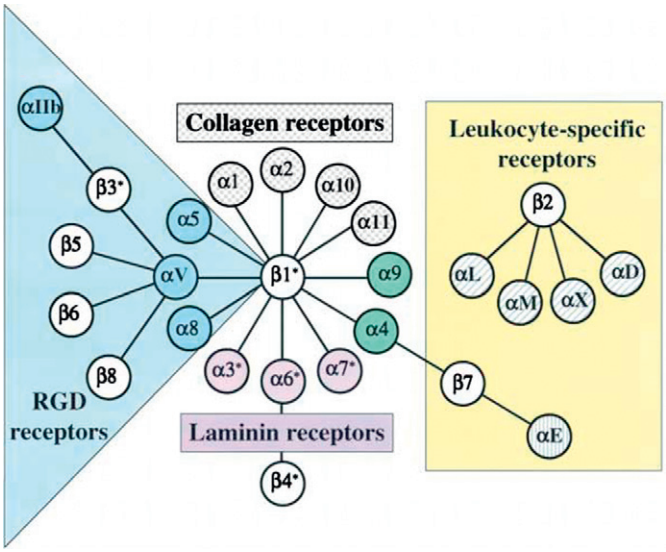

FIGURE 9-7 **Mammalian integrin receptors. Integrins are heterodimers.** Each subunit of the integrin heterodimer is a single-pass transmembrane protein with a large extracellular domain and a relatively short cytoplasmic domain. In the mammalian genome 8 β and 18 α subunits have been identified so far, and they are known to assemble into 24 distinct integrins. In the diagram shown here, each α and β subunit is represented as a circle, and the observed heterodimers are indicated by the lines drawn between them. The mammalian integrins can be separated into several subfamilies based on evolutionary relationships, ligand specificities and, in the case of β2 and β7 integrins, restricted expression in white blood cells. α subunits marked in gray and green are restricted to chordates, while all other α subunits are found throughout the metazoan kingdom and are therefore considered ancient. α subunits marked in gray have inserted I/A domains of approximately 200 amino acids, which serve a dominant ligand-binding function for these integrins. α subunits marked in purple interact specifically with the ECM protein laminin, while α subunits marked in blue interact with ECM proteins containing an exposed arginine-glycine-aspartate (RGD) peptide sequence. Asterisks denote subunits with alternatively spliced cytoplasmic domains. (From Hynes, 2002).

of αIIbβ3 integrin activation prevents pathological platelet aggregation and thrombosis. Similar to the αIIbβ3 integrin, many other integrins are not constitutively active. They can be and often are expressed on the cell surface in an inactive state that is characterized by a compact closed and bent conformation. Integrins in the activated high affinity ligand binding state are thought to be in an extended conformation. The transition from an inactive to an activated state requires long-range conformational changes, the exact nature of which is still unclear. However, studies analyzing the activation of β2 and β3 integrins revealed crucial roles for their cytoplasmic tails in regulating their affinities for their extracellular ligands. Based on these studies it is now thought that binding of intracellular activators, such as talin and kindlins, to highly conserved motifs within β cytoplasmic tails leads to a separation of the α and β cytoplasmic tails, a destabilization of interactions between the α and β transmembrane domains, and conformational changes in the extracellular domain. All these events combined result in the transformation of an inactive into an activated integrin with high ligand-binding affinity. In addition, talin binding can lead to the formation of active integrin clusters in the plane of the membrane and thus an increase in not only integrin affinity but also integrin surface density (valency). This dual effect is often referred to as an increase in integrin avidity. While talin binding seems essential for integrin activation it does not appear to be sufficient and thus requires co-activators such as the kindlins. However, the exact modes by which talin and kindlins cooperate are unclear.

Reciprocally to inside-out signaling, outside-in signaling induced by extracellular ligand binding leads to long range conformational changes that result in a separation of the α and β cytoplasmic tails and recruitment of proteins involved in establishing the integrin-cytoskeleton linkage. Interestingly, it is talin that is required for establishing this linkage. It is of note that the distinction between inside-out and outside-in signaling is conceptually useful. However, these two forms of integrin signaling more likely reflect the two sides of the same equilibrium, in which activation on the cytoplasmic side can enhance interactions at the extracellular side and vice versa.

Integrins regulate myelination

Myelination, the formation of a concentrically wrapped multi-lamellar sheet of insulating plasma membrane, the myelin sheath, around axonal segments, enables fast and efficient conduction of electrical impulses along axons. Myelin sheaths are produced by specialized glial cells, namely Schwann cells in the PNS and oligodendrocytes in the CNS.

In the PNS, myelinating glial cells express several integrins, in particular the laminin receptors α6β1, α6β4 and α7β1, for which *in vivo* roles in myelination have been characterized (Berti et al., 2006; Nodari et al., 2008; Van der Zee et al., 2008). In particular, deficits in myelination and/or myelin stability have been described in knockout mice lacking the β1 or β4 integrin subunit in Schwann cells. The ligands to these integrin receptors, namely laminins, are present in a thin sheet of organized ECM, the basal lamina, which surrounds myelinating Schwann cells but is not in contact with axons. The integrin α6β4 is expressed at the abaxonal surface, that is the basal lamina side, of myelinating Schwann cells and has been suggested to play an important role by anchoring Schwann cells to their basal lamina. During the process of active myelination, Schwann cells send out processes radially within axon bundles to segregate out axons destined to be myelinated and to obtain a one-to-one relationship with them. This process of radial sorting is severely impaired in knockout mice lacking the β1 subunit in Schwann cells. A similar phenotype is seen in laminin mutants, thus providing strong evidence for a pivotal role of interactions between laminins and a β1 subunit-containing integrin expressed by myelinating Schwann cells in radial sorting and PNS myelination (Berti et al., 2006; Colognato et al., 2005).

Oligodendrocytes, the myelinating glial cells of the CNS, also express a laminin receptor, namely α6β1 integrin. However, in contrast to PNS myelination the role of interactions between laminins and β1 subunit-containing integrins for CNS myelination is less well defined. In this regard, it is of note that traditional ECM-rich basal lamina structures are rare in the CNS. However, non-basal lamina laminin has been

INTEGRINS AS THERAPEUTIC TARGETS IN AUTOIMMUNE DISEASE

Babette Fuss

Cell adhesion refers to a molecular mechanism that is critical for normal development and maintenance of the nervous system. It is mediated by a wide variety of cell adhesion molecules, which have been subdivided into several families whose members share specific structure–function characteristics. Overall, it is the well-coordinated cooperation and cross-talk between cell adhesion molecules that ensures proper development and function of the nervous system. With that said, it is not surprising that there are nervous system diseases in which an increased expression of a cell adhesion molecule contributes to pathology and for which, consequently, an antibody directed specifically against the respective cell adhesion molecule may be converted into a therapeutic compound.

Anti-Integrin antibodies are used for the treatment of multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by progressive demyelination and neurodegeneration in the CNS (see Chapter 39, 'Diseases Involving Myelin'). Despite the existence of therapeutic approaches aimed at reducing inflammation, no curative treatment is currently available for MS. Thus, the need for better and more specific therapeutics is evident.

A key event in the pathophysiology of MS, and thus a potential target for therapeutic intervention, is the increased migration of lymphocytes into the CNS via mechanisms involving cell adhesion (Engelhardt, 2008). Lymphocyte entry into the inflamed CNS is thought to occur primarily via the blood–brain barrier (BBB), a structure consisting of endothelial cells that form intercellular tight junctions and are surrounded by a continuous basement membrane as well as astroglial end-feet. Under physiological conditions, the BBB restricts passage of circulating cells and macromolecules. Thus, only limited numbers of lymphocytes enter the CNS as part of routine immunosurveillance (Ransohoff et al., 2003). Under neuroinflammatory conditions as they exist in MS, however, the expression of certain chemokines and cell adhesion molecules is upregulated, leading to the presence of additional trafficking signals and to the entrance of significantly increased numbers of lymphocytes into the CNS by the following multi-step process.

First, lymphocytes interact with endothelial cells in a transient manner, a process referred to as tethering. Next, so-called lymphocyte rolling reduces the velocity of the cells' movement and allows lymphocytes to recognize the presence of chemokines that are presented on the endothelial cell surface. Both tethering and rolling involve cell adhesion molecules of the selectin family. Interaction of chemokines with their respective receptors present on the lymphocytes' cell surfaces activates intracellular signaling that leads to inside-out integrin activation, firm lymphocyte adhesion and ultimately lymphocytes crossing through the BBB by a process called diapedesis.

In the process of lymphocyte entry into the inflamed CNS the integrin $\alpha 4\beta 1$ was found to be crucial for both initial contact (tethering and rolling) and firm adhesion of lymphocytes to endothelial cells. Based on these findings, function blocking antibodies directed against the $\alpha 4$-integrin subunit were developed and found to inhibit inflammatory cell accumulation in the CNS in an animal model of MS (Yednock et al., 1992). For treatment of the human disease, a so-called humanized monoclonal antibody against the $\alpha 4$-integrin subunit was generated. In this humanized antibody, which is referred to as natalizumab and marketed as Tysabri, parts of high immunogenicity were eliminated to diminish recognition of the therapeutic and thus foreign antibody by the human immune system. Natalizumab provides the advantage of targeting a precise molecular mechanism and was thus tested in two large phase III clinical trials in which it was found to lead to significant improvement in clinical outcome for at least some types of MS patients. Despite growing evidence for potential complications due to adverse effects, natalizumab has been approved as the first immunospecific therapeutic for the treatment of certain forms of MS (Stuve et al., 2008).

The above-described example highlights the clinical relevance of research aimed at a better understanding of the role of cell adhesion molecules and the mechanisms they are involved in. In addition, it emphasizes the importance of continuing studies to further improve therapeutic strategies targeting cell adhesion mechanisms in general and in particular in MS.

References

Engelhardt, B. (2008). Immune cell entry into the central nervous system: Involvement of adhesion molecules and chemokines. *Journal of the Neurological Science*, *274*(1–2), 23–26.

Ransohoff, R. M., Kivisakk, P., & Kidd, G. (2003). Three or more routes for leukocyte migration into the central nervous system. *Nature Reviews Immunology*, *3*(7), 569–581.

Stuve, O., Gold, R., Chan, A., Mix, E., Zettl, U., & Kieseier, B. C. (2008). Alpha4-integrin antagonism with natalizumab: Effects and adverse effects. *Journal of Neurology*, *255*(Suppl. 6), 58–65.

Yednock, T. A., Cannon, C., Fritz, L. C., Sanchez-Madrid, F., Steinman, L., & Karin, N. (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. *Nature*, *356*(6364), 63–66.

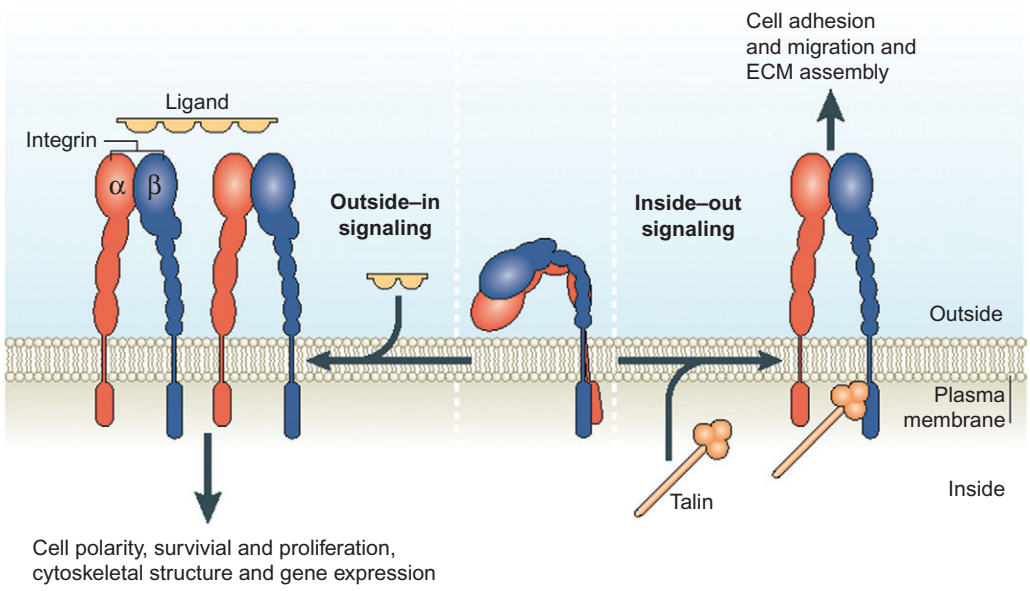

FIGURE 9-8 **Bidirectional integrin signaling. Integrins can signal in two directions.** During inside-out signaling, intracellular activators, such as talin or kindlins, bind to the β-integrin tail, leading to conformational changes that result in integrin activation. Integrins can also behave like traditional signaling receptors in transmitting information into cells by outside-in signaling. Binding of an integrin to its extracellular ligand changes the conformation of this integrin and leads to its activation. Current data favor a model in which integrin activation is associated with a conformational transition of the integrin's ectodomains from a closed and bent form to an open and extended form. However, the exact relationship between specific conformations and integrin activation remains controversial. In addition, inside-out and outside-in integrin signaling, even though conceptually segregated, are often closely linked. For example, integrin activation by inside-out signaling can increase ligand binding, resulting in outside-in signaling (From Shattil et al. 2010).

found present along myelinating axon tracts in the CNS, and recent studies suggest that during development the oligodendrocyte-expressed β1 subunit–containing integrin contributes to the regulation of CNS myelination (Barros et al., 2009; Camara et al., 2009). In addition to the α6β1 integrin, cells of the oligodendrocyte lineage express an array of αv containing integrins, namely αvβ1, αvβ3, αvβ5 and αvβ8 (Baron et al., 2005). Proposed functions of these αv containing integrins for cells of the oligodendrocyte lineage are derived primarily from cell culture studies and include the regulation of migration, proliferation and differentiation. Confirming these functions *in vivo* in the developing CNS has been difficult, and it appears that αv-containing integrins expressed by oligodendrocyte progenitor cells may play a more prominent role for repair of the myelin sheath under pathological conditions (Zhao et al., 2009).

COOPERATION AND CROSSTALK BETWEEN CELL ADHESION MOLECULES

Cell adhesion molecules are involved in important biological processes during nervous system development and maintenance. The diverse families of cell adhesion molecules that have been defined possess unique functional properties, which can be isolated in an experimental setting. In the context of a whole organism or tissue, however, it is the coordination of various cell adhesion molecules and the crosstalk between them that is crucial for the proper completion of the biological processes that cell adhesion molecules are functionally involved in (Chen & Gumbiner, 2006).

Various cell adhesion molecules cooperatively regulate the formation of interneuronal synapses in the CNS

Interneuronal synapses are highly specialized cell–cell junctions that provide the basis for the transfer of information throughout the CNS. They are formed in a precise fashion to ensure that interactions between neurons are limited to appropriate partners despite the presence of a large number of potential but inappropriate partners. Such selective synapse formation occurs during development in a tightly regulated and very reproducible manner, suggesting that this process is regulated, at least in part, by distinct molecular signals that mediate cell–cell interactions. Indeed, it is becoming increasingly clear that cell adhesion molecules play crucial roles in regulating the formation of fully functional synapses (Hortsch & Umemori, 2009).

Due to the significant heterogeneity of neuronal cell types in the CNS, that is, the presence of different classes of both excitatory and inhibitory neurons, deciphering the regulation of synapse formation is complicated. So far, CNS synapse formation has been primarily studied for excitatory glutamatergic synapses and is thought to occur in a multistep process (Fig. 9-9). In each of these steps a regulatory role of cell adhesion molecules has been proposed. The cell adhesion

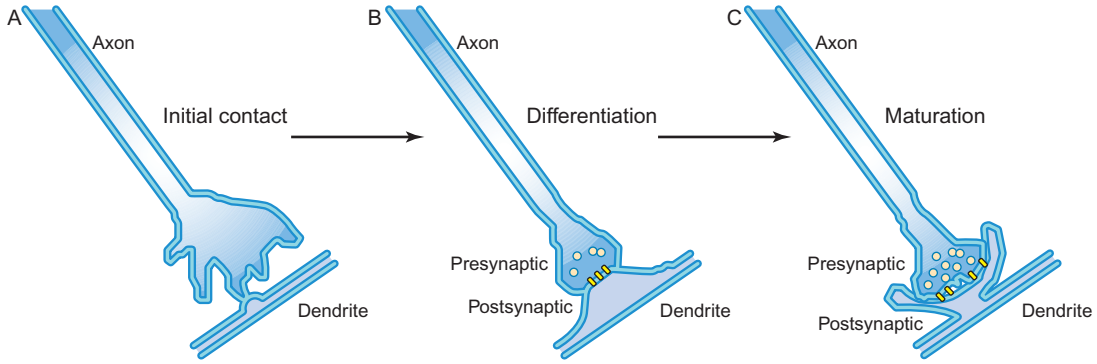

FIGURE 9-9 **CNS synapse formation: a multi-step process. (A)** Both axons and dendrites extend protrusions in search of the appropriate target. This recognition process is thought to involve several cell adhesion molecules including immunoglobulin superfamily members, cadherins and integrins. **(B)** After initial contact synapses differentiate to form a presynaptic side, which is characterized by the accumulation of synaptic vesicles (small circles), and a postsynaptic side. This differentiation is mediated by several cell adhesion molecules, such as neuroligins, neurexins and members of the immunoglobulin superfamily. **(C)** Further stabilization of the synaptic contact occurs during maturation, at the end of which a glutamatergic synapse has taken on a distinctive mushroom-like shape.

molecules implicated include various members of the immunoglobulin superfamily, cadherins, integrins, neurexins, neuroligins, synaptic adhesion-like molecules (SALMs), and leucine-rich repeat transmembrane neuronal proteins (LRRTMs) as well as ephrins and their receptors. Distinct functions for some of these cell adhesion molecules have been described. However, it is important to keep in mind that the functional role of a single cell adhesion molecule may be different for different types of neurons and thus different types of synapses. In addition, many cell adhesion molecules appear to be involved in a variety of aspects of synapse formation.

In the first step of synapse formation an initial contact between the tip of the axon, the axonal growth cone and the target cell must be established. This initial interaction, termed target selection, is highly specific. The large number of potential axon–target interactions, therefore, suggests the existence of an equally large number of molecular cues. In this regard, protocadherins are attractive candidates. They are present in considerable variety and expressed in specific patterns. Despite the existence of promising experimental data, however, their precise involvement in the regulation of synapse formation has yet to be determined (Yagi, 2008). More direct experimental evidence for target recognition by molecular matchmaking exists for neuron–neuron interactions within the developing retina. Four closely related members of the immunoglobulin superfamily are expressed at synaptic sites and by mutually exclusive subsets of interneurons and retinal ganglion cells, namely Sidekick-1, Sidekick-2, Down's syndrome cell adhesion molecule (Dscam) and Dscam-like-1 (DscamL). Loss- and gain-of-function studies indicate that the above four molecules, which engage in strict homophilic interactions, are critically involved in mediating selective connectivity between interneurons and retinal ganglion cells of the same subtype, that is, neurons expressing the same one of the above four molecules (Yamagata & Sanes, 2008). These findings support the idea that molecular cues direct initial target selection ultimately leading to the formation of highly precise neural circuits.

The second step in CNS synapse formation is characterized by the recruitment of pre- and postsynaptic components. Synaptic vesicles, the machinery for neurotransmitter release, accumulate at the presynaptic site, while receptors for the respective neurotransmitters and their associated downstream signaling scaffolds are assembled at the postsynaptic site. Perhaps the best-known cell adhesion molecule system involved in regulating synaptic differentiation and function is the neurexin–neuroligin system (Fig. 9-10 and Craig & Kang, 2007; Sudhof, 2008). Neurexins are located on the presynaptic site. They are derived in the mammalian genome from three genes, each of which gives rise to an α-neurexin and a β-neurexin via the use of independent promoters. In addition, extensive alternative splicing generates thousands of potential neurexin isoforms with diverse ectodomains. Neuroligins are endogenous ligands to both α- and β-neurexins and they are located on the postsynaptic site. Neuroligins are derived from four genes in rodents and from five genes in humans. Alternative splicing is used to generate additional neuroligin variants. The specificity of neurexins–neuroligin complex formation at the synapse appears to depend on which principal genes are expressed and which splice variants are used. Interestingly, different isoforms of neurexins and neuroligins selectively contribute to either excitatory or inhibitory synapse development. First evidence for a significant functional role of neurexins and neuroligins in synapse formation came from studies in which these proteins were artificially expressed in non-neuronal cells. Notably, neuroligin-expressing non-neuronal cells were found to increase the number of presynaptic specializations in co-cultured neurons (Scheiffele et al., 2000). In subsequent complementary experiments it was shown that neurexin-expressing non-neuronal cells can increase the number of postsynaptic specialization on co-cultured neurons.

One could conclude from these studies that neurexin–neuroligin interactions play a role in the initial contact between appropriate neuronal partners. However, gene knockout analyses suggest otherwise. For example, mice lacking one of the neuroligin genes have nearly normal synapse numbers with an apparently normal ultrastructure, but synapse function is

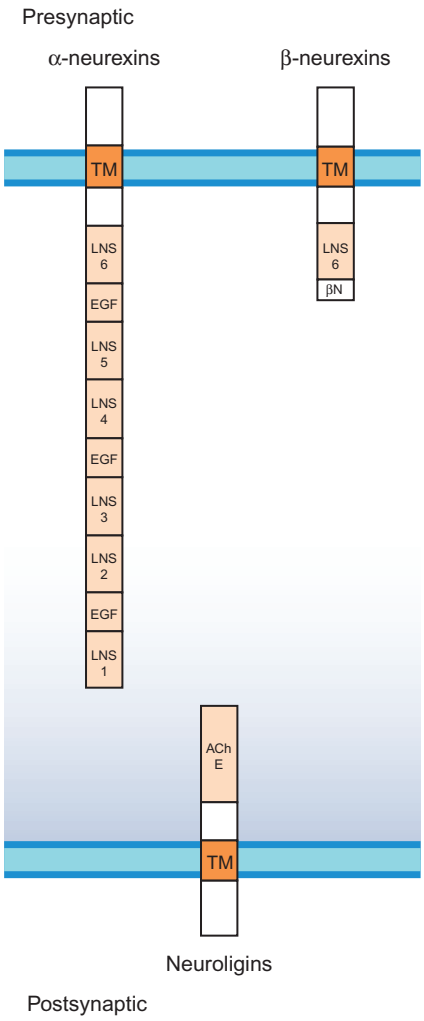

FIGURE 9-10 **Domain structures of neurexins and neuroligins. Neurexins are presynaptic cell adhesion molecules that in mammals are derived from three genes.** Each neurexin gene generates a larger α-neurexin and a smaller β-neurexin isoform via the use of an upstream and downstream promoter, respectively. α-neurexins possess three extracellular neurexin repeats, which are each composed of two LNS (laminin, neuroexin, sex hormone-binding globulin) domains flanking an intercalated EGF (epidermal growth factor)-like domain. β neurexins contain a small N-terminal β-neurexin-specific sequence (βN) and a single extracellular LNS domain. Neuroligins are postsynaptic cell adhesion molecules that are derived from four genes in rodents and from five genes in humans. Neuroligins contain a large extracellular domain with homology to acetylcholinesterase (AChE), which lacks cholinesterase activity but mediates binding to both α- and β-neurexins. Both neurexins and neuroligins have relatively short intracellular domains, which are thought to mediate intracellular linkage with other synaptic proteins.

severely impaired. Thus, the picture that is currently emerging is that the formation of specific neurexin–neuroligin complexes may primarily contribute to synapse function. Notably, mutations in the genes encoding neurexins and neuroligins have been associated with mental retardation and autism spectrum disorders. These clinical correlations further support functional roles for neurexins and neuroligins in regulating highly precise neural circuits, and they may provide a link between synaptic cell adhesion systems and cognition.

Although neurexins and neuroligins contribute to the formation of fully functional synapses, they are clearly not the only cell adhesion system involved in synaptic differentiation. For example, the immunoglobulin superfamily member known as synaptic cell adhesion molecule 1 (SynCAM 1, alternatively named Tslc1 and nectin-like protein 2) is, similar to neuroligin, capable of inducing presynaptic assembly in co-cultured neurons when expressed in non-neuronal cells. Four SynCAM genes sharing the same domain organization have been identified, and each SynCAM exhibits a distinct spatiotemporal expression pattern in the brain (Thomas et al., 2008). Functionally, SynCAMs have been implicated in affecting the organization and function of synapses primarily via heterophilic interactions with each other (Fogel et al., 2007).

The third step in synapse formation is a prolonged maturation phase, during which some synapses are stabilized while others are eliminated. One of the most dramatic examples of synapse maturation is the transformation of an immature glutamatergic synapse into a mature mushroom-shaped so-called dendritic spine. This transformation is associated with significant remodeling of the actin cytoskeleton, and several cell adhesion molecules have been implicated in the cooperative regulation of this process. Central molecular players in this regulation are thought to be nectins, members of the immunoglobulin superfamily. There are four nectin family members, all of which can interact in a *trans* homophilic as well as a *trans* heterophilic manner with nectins. The short cytoplasmic domain of nectins binds to afadin, an actin-binding protein providing a direct link to the cytoskeleton. Based on existing studies investigating the role of nectins in the nervous system, and by analogy to the extensively studied role of nectins in adherens junctions of epithelial cells, the following scenario for the role of nectins in synapse formation is currently developing. Nectin-1 and -3 are localized asymmetrically at the pre- and postsynaptic sites, respectively. Upon initial contact, they form trans nectin-1–nectin-3 interactions, a process that is followed by the recruitment of N-cadherin. Nectins and N-cadherin colocalize, and they both are associated intracellularly with proteins that provide a link to the cytoskeleton, afadin and β-catenin, respectively. In addition, nectins activate the small G-proteins Cdc42 and Rac1, which regulate the cytoskeleton via their downstream effectors. Thus, the cooperative action of nectins and cadherins represents an attractive mechanism for regulating synapse shape via the reorganization of the cytoskeleton. In this regard it is of note that in hippocampal neurons isolated from nectin-1 knockout mouse dendritic spines are unusually elongated and deformed.

Integrin-cadherin cross-talk regulates neurite outgrowth

During development neuronal cell bodies extend elongated protrusions termed neurites that will ultimately differentiate into axons or dendrites. Axons frequently grow over long distances in order to make very precise connections with

an appropriate target. Despite the complexity of the nervous system, where over 10^{12} axons must find their targets, the pattern of axonal outgrowth for any one axon is highly reproducible from one individual to another. This specificity of axonal outgrowth is dependent on the sensorimotor properties of the tip of the growing axon, the growth cone. It is the growth cone that recognizes the molecular cues present in the environment and integrates the information to determine the pathway the growing axon will take. This response process requires both cell adhesion and subsequent triggering of an intracellular signaling cascade. A significant fraction of the molecular cues that the growth cone encounters are cell adhesion molecules. These are expressed by non-neuronal cells, for example glia cells, or older axons that have already traversed that particular pathway. In addition, growth cones encounter ECM-derived signals, which they interpret largely via the use of integrins. It is of note that once interpreted, the extracellular environment can be of either outgrowth-promoting or inhibitory nature (Kiryushko et al., 2004; Tessier-Lavigne & Goodman, 1996).

Integrins, in particular β1-integrins, and cadherins, in particular N-cadherin, have been both implicated in promoting neurite outgrowth. In the context of the developing nervous system, it appears reasonable to assume that the activity of these two neurite outgrowth-promoting adhesion systems needs to be well coordinated. Experimental support for a cross-regulation of integrin function and cadherin adhesion activity comes primarily from studies investigating epithelial–mesenchymal transition during embryonic development (Marsden & DeSimone, 2003). However, some evidence exists for a similar cross-talk in the regulation of neurite outgrowth. In studies using retina-derived explant cultures as an experimental model for investigating neurite outgrowth, a cell-permeable peptide mimicking the juxtamembrane region of the cytoplasmic domain of N-cadherin was found to not only inhibit N-cadherin function but to also inhibit β1-integrin function and neurite outgrowth (Arregui et al., 2000). The effect on β1-integrin function was found to be dependent on the expression of N-cadherin and to be independent of transcription or translation, demonstrating that the effect of the N-cadherin-specific peptide on integrin function indeed acts through N-cadherin. Such cross-talk could involve membrane-proximal protein–protein interactions and/or downstream effectors of signaling pathways. In the case of the above example, cross-talk between β1-integrin and N-cadherin seems primarily mediated by intracellular effectors able to regulate both integrins and cadherins. The above findings provide promising support for a role for integrin–cadherin crosstalk in the regulation of axon outgrowth. However, additional studies will be necessary to further substantiate this idea.

SUMMARY

Cell adhesion molecules are indispensable for nervous system development and maintenance. They mediate cell-to-cell and/or cell-to-ECM interactions, and by doing so they trigger intracellular responses affecting intracellular signaling, cytoskeletal organization and/or gene expression. Three major so called 'superfamilies' of cell adhesion molecules have been characterized in the CNS, namely IgCAMs, cadherins and integrins. Distinct functions for individual cell adhesion molecules can be identified. However, in the context of the developing and mature nervous system, it is more likely the temporally and spatially controlled net result of all cell adhesion molecule interactions occurring at the cell surface that determine a cellular response.

References

Arregui, C., Pathre, P., Lilien, J., & Balsamo, J. (2000). The nonreceptor tyrosine kinase Fer mediates crosstalk between N-cadherin and beta1-integrins. *Journal of Cell Biology*, *149*(6), 1263–1274.

Baron, W., Colognato, H., & ffrench-constant, C. (2005). Integrin-growth factor interactions as regulators of oligodendroglial development and function. *Glia*, *49*(4), 467–479.

Barros, C. S., Nguyen, T., Spencer, K. S., Nishiyama, A., Colognato, H., & Muller, U. (2009). Beta1 integrins are required for normal CNS myelination and promote AKT-dependent myelin outgrowth. *Development*, *136*(16), 2717–2724.

Berti, C., Nodari, A., Wrabetz, L., & Feltri, M. L. (2006). Role of integrins in peripheral nerves and hereditary neuropathies. *Neuromolecular Medicine*, *8*(1–2), 191–204.

Blaschuk, O. W., & Devemy, E. (2009). Cadherins as novel targets for anti-cancer therapy. *European Journal of Pharmacology*, *625*(1–3), 195–198.

Boggon, T. J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B. M., & Shapiro, L. (2002). C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science*, *296*(5571), 1308–1313.

Bork, P., Holm, L., & Sander, C. (1994). The immunoglobulin fold. Structural classification, sequence patterns and common core. *Journal of Molecular Biology*, *242*(4), 309–320.

Bradley, J. E., Ramirez, G., & Hagood, J. S. (2009). Roles and regulation of Thy-1, a context-dependent modulator of cell phenotype. *Biofactors*, *35*(3), 258–265.

Camara, J., Wang, Z., Nunes-Fonseca, C., Friedman, H. C., Grove, M., Sherman, D. L., et al. (2009). Integrin-mediated axoglial interactions initiate myelination in the central nervous system. *Journal of Cell Biology*, *185*(4), 699–712.

Chen, X., & Gumbiner, B. M. (2006). Crosstalk between different adhesion molecules. *Current Opinion in Cell Biology*, *18*(5), 572–578.

Clegg, D. O., Wingerd, K. L., Hikita, S. T., & Tolhurst, E. C. (2003). Integrins in the development, function and dysfunction of the nervous system. *Frontiers in Bioscience*, *8*, d723–750.

Colognato, H., ffrench-Constant, C., & Feltri, M. L. (2005). Human diseases reveal novel roles for neural laminins. *Trends in Neuroscience*, *28*(9), 480–486.

Craig, A. M., & Kang, Y. (2007). Neurexin–neuroligin signaling in synapse development. *Current Opinion in Neurobiology*, *17*(1), 43–52.

Crocker, P. R., Paulson, J. C., & Varki, A. (2007). Siglecs and their roles in the immune system. *Nature Reviews Immunology*, *7*(4), 255–266.

Delon, I., & Brown, N. H. (2007). Integrins and the actin cytoskeleton. *Current Opinion in Cell Biology*, *19*(1), 43–50.

Ditlevsen, D. K., & Kolkova, K. (2010). Signaling pathways involved in NCAM-induced neurite outgrowth. *Advances in Experimental Medicine and Biology*, *663*, 151–168.

Edelman, G. M. (1987). CAMs and Igs: Cell adhesion and the evolutionary origins of immunity. *Immunological Reviews*, *100*, 11–45.

Fogel, A. I., Akins, M. R., Krupp, A. J., Stagi, M., Stein, V., & Biederer, T. (2007). SynCAMs organize synapses through heterophilic adhesion. *Journal of Neuroscience*, *27*(46), 12516–12530.

Geiger, B., & Ayalon, O. (1992). Cadherins. *Annual Review of Cell Biology*, *8*, 307–332.

Gumbiner, B. M. (2005). Regulation of cadherin-mediated adhesion in morphogenesis. *Nature Reviews Molecular Cell Biology*, *6*(8), 622–634.

Hildebrandt, H., Muhlenhoff, M., & Gerardy-Schahn, R. (2010). Polysialylation of NCAM. *Advances in Experimental Medicine and Biology*, *663*, 95–109.

Hortsch, M., & Umemori, H. (Eds.). (2009). *The sticky synapse*. New York, NY: Springer.

Hulpiau, P., & van Roy, F. (2009). Molecular evolution of the cadherin superfamily. *International Journal of Biochemistry and Cell Biology*, *41*(2), 349–369.

Hynes, R. O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell*, *110*(6), 673–687.

Jeanes, A., Gottardi, C. J., & Yap, A. S. (2008). Cadherins and cancer: How does cadherin dysfunction promote tumor progression? *Oncogene*, *27*(55), 6920–6929.

Kiryushko, D., Berezin, V., & Bock, E. (2004). Regulators of neurite outgrowth: Role of cell adhesion molecules. *Annals of the New York Academy of Science*, *1014*, 140–154.

Maness, P. F., & Schachner, M. (2007). Neural recognition molecules of the immunoglobulin superfamily: Signaling transducers of axon guidance and neuronal migration. *Nature Neuroscience*, *10*(1), 19–26.

Marsden, M., & DeSimone, D. W. (2003). Integrin–ECM interactions regulate cadherin–dependent cell adhesion and are required for convergent extension in Xenopus. *Current Biology*, *13*(14), 1182–1191.

Milner, R., & Campbell, I. L. (2002). The integrin family of cell adhesion molecules has multiple functions within the CNS. *Journal of Neuroscience Research*, *69*(3), 286–291.

Morishita, H., & Yagi, T. (2007). Protocadherin family: Diversity, structure, and function. *Current Opinion in Cell Biology*, *19*(5), 584–592.

Murphy, K. M., Travers, P., & Walport, M. (Eds.), (2007). *Janeway's immunobiology* (7th ed.). New York, NY: Garland Science.

Nodari, A., Previtali, S. C., Dati, G., Occhi, S., Court, F. A., Colombelli, C., et al. (2008). Alpha6beta4 integrin and dystroglycan cooperate to stabilize the myelin sheath. *Journal of Neuroscience*, *28*(26), 6714–6719.

Nollet, F., Kools, P., & van Roy, F. (2000). Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. *Journal of Molecular Biology*, *299*(3), 551–572.

Pasterkamp, R. J., & Giger, R. J. (2009). Semaphorin function in neural plasticity and disease. *Current Opinion in Neurobiology*, *19*(3), 263–274.

Pokutta, S., & Weis, W. I. (2007). Structure and mechanism of cadherins and catenins in cell–cell contacts. *Annual Review of Cell and Developmental Biology*, *23*, 237–261.

Redies, C. (2000). Cadherins in the central nervous system. *Progress in Neurobiology*, *61*(6), 611–648.

Ruoslahti, E. (1996). RGD and other recognition sequences for integrins. *Annual Review of Cell and Developmental Biology*, *12*, 697–715.

Sakisaka, T., & Takai, Y. (2005). Cell adhesion molecules in the CNS. *Journal of Cell Science*, *118*(Pt 23), 5407–5410.

Scheiffele, P., Fan, J., Choih, J., Fetter, R., & Serafini, T. (2000). Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell*, *101*(6), 657–669.

Shapiro, L., Love, J., & Colman, D. R. (2007). Adhesion molecules in the nervous system: Structural insights into function and diversity. *Annual Review of Neuroscience*, *30*, 451–474.

Shattil, S. J., Kim, C., & Ginsberg, M. H. (2010). The final steps of integrin activation: The end game. *Nature Reviews Molecular Cell Biology*, *11*(4), 288–300.

Smith, D. K., & Xue, H. (1997). Sequence profiles of immunoglobulin and immunoglobulin-like domains. *Journal of Molecular Biology*, *274*(4), 530–545.

Sudhof, T. C. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature*, *455*(7215), 903–911.

Suzuki, S. C., & Takeichi, M. (2008). Cadherins in neuronal morphogenesis and function. *Development, Growth and Differentiation*, *50*(Suppl. 1), S119–130.

Takeichi, M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*, *251*(5000), 1451–1455.

Takeichi, M. (2007). The cadherin superfamily in neuronal connections and interactions. *Nature Reviews Neuroscience*, *8*(1), 11–20.

Tessier-Lavigne, M., & Goodman, C. S. (1996). The molecular biology of axon guidance. *Science*, *274*(5290), 1123–1133.

Thomas, L. A., Akins, M. R., & Biederer, T. (2008). Expression and adhesion profiles of SynCAM molecules indicate distinct neuronal functions. *Journal of Comparative Neurology*, *510*(1), 47–67.

Van der Zee, C. E., Kreft, M., Beckers, G., Kuipers, A., & Sonnenberg, A. (2008). Conditional deletion of the Itgb4 integrin gene in Schwann cells leads to delayed peripheral nerve regeneration. *Journal of Neuroscience*, *28*(44), 11292–11303.

Williams, A. F., & Barclay, A. N. (1988). The immunoglobulin superfamily domains for cell surface recognition. *Annual Review of Immunology*, *6*, 381–405.

Yagi, T. (2008). Clustered protocadherin family. *Development, Growth and Differentiation*, *50*(Suppl. 1), S131–140.

Yamagata, M., & Sanes, J. R. (2008). Dscam and sidekick proteins direct lamina-specific synaptic connections in vertebrate retina. *Nature*, *451*(7177), 465–469.

Yamaguchi, Y. (2000). Lecticans: Organizers of the brain extracellular matrix. *Cellular and Molecular Life Sciences*, *57*(2), 276–289.

Ypsilanti, A. R., Zagar, Y., & Chédotal, A. (2010). Moving away from the midline: New developments for Slit and Robo. *Development*, *137*(12), 1939–1952.

Zaidel-Bar, R., & Geiger, B. (2010). The switchable integrin adhesome. *Journal of Cell Science*, *123*(Pt 9), 1385–1388.

Zelensky, A. N., & Gready, J. E. (2005). The C-type lectin-like domain superfamily. *FEBS Journal*, *272*(24), 6179–6217.

Zhao, C., Fancy, S. P., Franklin, R. J., & ffrench-Constant, C. (2009). Up-regulation of oligodendrocyte precursor cell alphaV integrin and its extracellular ligands during central nervous system remyelination. *Journal of Neuroscience Research*, *87*(15), 3447–3455.