HHS Public Access

Author manuscript

Nat Rev Neurosci. Author manuscript; available in PMC 2016 February 17.

Published in final edited form as:

Nat Rev Neurosci. 2007 March ; 8(3): 206–220. doi:10.1038/nrn2075.

Cell adhesion molecules: signalling functions at the synapse

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Abstract

Many cell adhesion molecules are localized at synaptic sites in neuronal axons and dendrites. These molecules bridge pre- and postsynaptic specializations but do far more than simply provide a mechanical link between cells. In this review, we will discuss the roles these proteins have during development and at mature synapses. Synaptic adhesion proteins participate in the formation, maturation, function and plasticity of synaptic connections. Together with conventional synaptic transmission mechanisms, these molecules are an important element in the trans-cellular communication mediated by synapses.

CNS synapses are specialized sites of cell–cell contact that mediate the transmission of information between neurons. Synapses are a key site of regulation within neural networks and are characterized by multi-protein complexes arranged at tightly apposed pre- and post-synaptic terminals. Communication between neurons at synapses is mediated primarily by neurotransmitter release and by the gating of postsynaptic receptor ion channels, but a growing body of evidence indicates that signalling is also mediated by adhesion molecules that interact in a homo- or heterophilic fashion across the synaptic cleft. As at other cell–cell junctions, such as epithelial tight junctions or the immune synapse, research in a variety of neuronal subtypes has shown that synaptically localized cell adhesion molecules (SAMs) are not merely static structural components but are often dynamic regulators of synapse function. When pairs of SAMs interact, they can induce the formation of new synapses or modulate the function of existing synapses through signalling cascades or secondary protein–protein interactions. Numerous studies indicate that interactions between specific SAMs can control synapse formation, regulate dendritic spine morphology, modify synaptic receptor function and modulate synaptic plasticity. So, SAMs can mediate physical interactions between cells and act at multiple steps in the life of a synapse (FIG. 1).

Here, we focus on well-studied classes of SAMs that have roles at both developing and mature synapses, namely neurexins and neuroligins, EphBs and ephrin-Bs, immunoglobulin

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Competing interests statement

The authors declare no competing financial interests.

Note added in proof

In a recently published manuscript, Futai *et al.*[144] demonstrate that trans-synaptic β-neurexin–neuroligin interactions can modulate presynaptic function. The authors show that overexpression or knockdown of PSD-95 in the postsynaptic cell modulates presynaptic release probability, and that these effects are blocked when trans-synaptic β-neurexin–neuroligin signalling is disrupted. This paper provides further evidence that SAMs act to regulate synaptic function.

(Ig)-containing cell adhesion molecules and cadherins. We discuss the ways in which these proteins seem to control discrete aspects of synaptic development or function through specialized motifs that either induce downstream signalling or recruit specific sets of proteins. Importantly, SAMs share common protein domains that can act in a similar fashion to coordinate synapse development, but also have unique motifs that endow each protein with distinct functions. As yet, no single pair of SAMs seems to be sufficient to organize all aspects of synapse development, indicating that SAMs might have overlapping functions or act together at synaptic sites. One intriguing possibility is that the presence of particular sets of these molecules at synaptic sites might serve to specify certain classes or types of synapses. In the complex process of synaptic plasticity, SAMs appear to function primarily as modulators, and in some cases the action of specific SAMs is restricted to certain synapses.

Cell adhesion and synaptogenesis

Synapse formation requires the assembly of a highly ordered protein complex containing receptors, signalling molecules and scaffolding proteins. Various signals including neurotransmission, soluble factors secreted by neurons or glia, and direct cell–cell contacts have roles in synaptic development[1–3]. However, cell–cell adhesion interactions are particularly attractive candidate mediators of synaptogenesis because of their potential to bidirectionally coordinate molecular and morphological synapse differentiation (TABLE 1).

Neurexins and neuroligins

Perhaps the most extensively described trans-synaptic signal involved in synaptogenesis is the interaction between presynaptic neurexins and postsynaptic neuroligins, which act as calcium-dependent cell adhesion molecules[4–7]. There are three neurexin genes (*Nrxn1–3*), each of which encodes two transcripts, α- and β-neurexin, through the use of two distinct promoters[8]. α- and β-neurexin share identical intracellular domains[9], but α-neurexin has a larger extracellular domain that might allow it to take part in distinct extracellular interactions[9]. Alternative splicing results in more than 1000 neurexin isoforms, raising the possibility that different neurexin splice variants encode for specificity in synaptic connections[8,10,11]. Neuroligins (neuroligin 1–3) are expressed predominantly in the brain and bind neurexins via an extracellular esterase-like domain. Neuroligins undergo alternative splicing, which promotes binding to either α- or β-neurexin[12,13] (FIG. 2). Both neurexins and neuroligins also contain an intracellular PDZ binding domain that mediates interactions with synaptic scaffolding proteins[14–16]. So, based on structure and splicing, neurexins and neuroligins are equipped to take part in determining the specificity and differentiation of synaptic contacts.

A number of studies have demonstrated a role for neurexins and neuroligins in both excitatory and inhibitory synapse formation *in vitro*. Overexpression and knockdown of neuroligins results in an increase and decrease, respectively, in synapse number[17–20]. These manipulations also result in changes in dendritic spine density[17], although the underlying mechanism remains to be determined. In addition, neuroligin-expressing non-neuronal cells co-cultured with neurons induce pre-synaptic differentiation of contacting axons[20,21], whereas the expression of neurexins in non-neuronal cells causes clustering of postsynaptic

proteins in contacting den-drites[13,20,22]. Therefore, *in vitro* neurexin–neuroligin interactions can organize components of both pre- and postsynaptic terminals.

The control of inhibitory or excitatory synapse formation by neurexin–neuroligin interactions is guided by the different neurexin and neuroligin subtypes involved, in combination with extracellular domain splice variants. Neuroligin 1 is primarily localized to excitatory synapses[7] and promotes the formation of excitatory specializations[17,20], although this depends on alternative splicing (FIG. 2). By contrast, neuroligin 2 is localized to inhibitory synapses[23] and preferentially induces the formation of inhibitory contacts[17,20]. Although neuroligin 3 localization is less clear, it seems to promote excitatory synaptogenesis[17,20]. In addition to this specification of function based on neuroligin localization within a neuron, an extracellular domain splice code guides the binding of particular neuroligin and neurexin variants, thereby determining whether the interaction promotes excitatory or inhibitory synapse formation[12,13,24] (FIG. 2). These splice-dependent interactions have also been implicated in the differential regulation of synapse formation versus expansion[12]. Interestingly, knockdown of all three neuroligins by small interfering RNA (siRNA) in cultured neurons results in decreased frequency of inhibitory, but not excitatory, miniature synaptic currents[17], raising the possibility that neuroligin signalling is particularly important for inhibitory synaptogenesis *in vitro*.

The extracellular splice insert domains of neurexin and neuroligin are not the only motifs of these proteins with specific functions during synaptogenesis. Neurexins bind the presynaptic scaffold molecules calcium/calmodulin-dependent serine protein kinase (CASK) and MINT (Munc 18 interacting protein; lin-10/X11) through PDZ binding domain interactions, which couple neurexin signalling to synaptic vesicle exocytosis[15,16,25] and link neurexin signalling to changes in the actin cytoskeleton through protein 4.1 (REF. 26). The synaptogenic activity of neuroligins depends on lateral (*cis*) clustering of neuroligin molecules, which requires the presence of particular amino acids located in the neuroligin esterase-like ectodomain[27]. This multimerized neuroligin complex then binds and clusters neurexins, leading to the recruitment of presynaptic machinery[27]. Furthermore, through intracellular PDZ binding domain interactions, neuroligins bind the postsynaptic scaffold molecule PSD-95 (postsynaptic density protein-95) (REF. 14), an important component of the postsynaptic density that clusters other postsynaptic proteins[28–31]. NMDARs (*N*-methyl-D-aspartate receptors) and presynaptic specializations are also recruited to neuroligin-induced synapses[17,20], although this is independent of the interaction between neuroligin and PSD-95 (REF. 17). An additional factor, such as neuronal activity, is required to recruit AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) to these postsynaptic sites[22]. The intracellular interaction of neuroligins with PSD-95 also seems to be important for determining neuroligin distribution to excitatory or inhibitory synapses, and therefore the excitation/inhibition (E/I) balance in neurons[18,19]. Finally, co-clusters of neuroligin 1 and PSD-95 can serve as predetermined ‘hotspots’ to which presynaptic machinery is recruited[31], indicating that this interaction might be involved in determining where on a neuron synapses form. These results provide evidence that specific domains of both the intracellular and extra-cellular portions of neuroligins and neurexins control particular synaptogenic functions *in vitro* (FIG. 3).

In vivo loss-of-function studies show that both neuroligins and α-neurexins are essential genes (TABLE 2). Mice lacking neuroligins 1–3 die shortly after birth due to respiratory failure[32]. Surprisingly, although glutamatergic and GABA (γ-aminobutyric acid)-mediated neurotransmission are impaired in brainstem respiratory centres in these animals, there is no significant reduction in synapse number[32]. So, the removal of neuroligins results in perturbations in synapse maturation causing reduced neurotransmission, but does not cause a substantial loss of either excitatory or inhibitory synaptic contacts. Interestingly, a shift in the balance between excitation and inhibition in the respiratory brainstem occurs in neuroligin-knockout animals[32], and genetic mutations in neuroligins have been associated with autism spectrum disorders, in which disturbances to the E/I balance have been implicated[33–35]. While the phenotype of mice lacking β-neurexins is not yet known, mice lacking α-neurexins have a dysfunction in synaptic vesicle exocytosis and die after birth[36]. Ultrastructural analysis of the brains of α-neurexin-knockout mice has revealed a specific decrease in inhibitory synapse density, although it is not clear whether this loss is due to the absence of α-neurexins or results from abnormalities in synaptic transmission[36]. Taken together, *in vivo* data indicates that this adhesion system is an important regulator of synaptic maturation and function.

Although the central finding regarding the importance of neuroligins in the regulation of E/I balance is consistent between work *in vitro* and *in vivo*, the recent *in vivo* results contrast with the *in vitro* data that suggests an inductive function for neuroligins during synaptogenesis[17,20,21]. Although what might account for this difference is unknown, single-cell knockdown of neuroligins in otherwise wild-type cultures might result in activity-dependent homeostatic effects that lead to reduced numbers of synapses. The absence of a defect in synapse formation in homotypic neuroligin triple-null cultures is consistent with this idea. Alternatively, these differences might reflect compensation by other synaptogenic factors or redundancy in the systems that control synapse formation. Regardless, these findings indicate that neuroligins can trigger synaptogenesis *in vitro* but are not essential for this process *in vivo*, and point towards a significant role for neurexin/neuroligin interactions in the later stages of both excitatory and inhibitory synapse development.

EphBs and ephrin-Bs

Ephs are a family of 13 receptor tyrosine kinases that are divided into A and B subclasses based on their affinity for the ephrin-A or ephrin-B ligands[37]. Both subclasses of ephrin are membrane-attached: ephrin-As are bound by glycosylphosphatidylinositol (GPI) linkage and ephrin-Bs are transmembraneous. We focus here on the role of EphB–ephrin-B interactions in synaptogenesis because the preponderance of data implicates these subtypes, although signalling between neuronal EphAs and glial ephrin-As has also been shown to modify dendritic spine morphogenesis[38], and EphA7 receptors are found at the postsynaptic density in the hippocampus[39]. Ephrin-B binding to EphBs results in bidirectional signalling between the receptor- and ligand-containing cells, thereby permitting contact-mediated trans-cellular signalling. EphBs have a number of protein domains — including a PDZ binding domain, a kinase domain and juxtamembrane tyrosines — that mediate their ability to induce signalling in a number of down-stream pathways, such as activation of non-receptor tyrosine kinases and activation of GTPases through particular guanine nucleotide exchange

factors (GEFs)[40]. Although ephrin-B signalling is less well defined, these ligands also contain a PDZ binding domain and tyrosine phosphorylation sites, and, following Src-mediated phosphorylation, can signal downstream through adaptor proteins[40,41].

EphBs and ephrin-Bs have classic functions in axon guidance and boundary formation throughout early development[40]. An additional role for these molecules at synapses was first suggested by the observations that EphBs and ephrin-Bs are present at synaptic sites in the CA1 region of the hippocampus and in hippocampal cultures[39,42]. Localization experiments along with functional studies indicate that EphB2 is primarily found in postsynaptic terminals in the hippocampus and cortex during synaptogenesis[39,42–44], whereas ephrin-B expression patterns are more complex. Depending on the brain area and ephrin-B subtype, ephrin-Bs can be found in both pre- and postsynaptic terminals[44–48]. So, in addition to their role as presynaptic ligands for EphBs, ephrin-Bs might also directly modify postsynaptic organization or function.

Several studies indicate that postsynaptic EphBs control multiple aspects of excitatory synaptogenesis. Clustering and activation of EphB with a soluble ephrin-B–Fc fusion protein induces the formation of dendritic spines, clustering of NMDARs and AMPARs, and increased numbers of presynaptic terminals[43,44,49,50]. Knockdown of EphB2 in cortical neurons results in decreased presynaptic specializations, spines and functional excitatory synaptic inputs; non-neuronal cells expressing EphB2 trigger presynaptic differentiation of contacting axons in co-cultured neurons[50]. Finally, the importance of EphBs in synapse formation *in vitro* is highlighted by the almost complete loss of excitatory postsynaptic specializations and dendritic spines in cultured hippocampal neurons from mice lacking EphB1–3 (REF. 44).

Particular domains of postsynaptic EphBs and specific downstream signalling pathways seem to control distinct aspects of synaptogenesis (FIG. 3). Ephrin-B binding to EphB2 induces a direct interaction between the extracellular domains of the NMDAR and EphB2 (REF. 43), and the ability of EphB2 to trigger presynaptic differentiation requires its ephrin-binding globular domain[50]. The intracellular domain of EphB2 has been found to interact and co-cluster with AMPARs through PDZ binding domain interactions[46,50], which also leads to enhanced AMPAR surface retention in neurons[50]. The kinase activity of EphB mediates its ability to induce dendritic spine morphogenesis by signalling through various Rho GTPases. Activation of EphB results in the EphB kinase-dependent phosphorylation of the Rho GEF kalirin and downstream signalling to Rac1 and P21-activated kinase (PAK)[49]. Treatment with ephrin-B–Fc also results in the phosphorylation of syndecan 2, a known regulator of dendritic spine morphogenesis[51,52]. Phosphorylation of syndecan 2 induces the recruitment and increased activation of the GEF intersectin, leading to upregulated activity of the Rho GTPase Cdc42, which triggers actin polymerization[51,53]. Finally, application of ephrin-B–Fc induces the association of focal adhesion kinase (FAK), growth factor receptor-bound protein 2 (GRB2) and Src with EphB2, as well as increasing the association of FAK with paxillin[54]. Activated Src phosphorylates FAK, which activates RhoA and shortens dendritic filopodia. So, *in vitro*, EphB interactions can control both pre- and postsynaptic differentiation through the activities of particular protein domains. Future work will be

required to understand whether EphB acts primarily as an inductive or maturation factor during synapse development.

In vivo evidence offers further support for the role of EphBs in excitatory synaptogenesis (TABLE 3). Mice lacking EphB2 show a 40% reduction in the number of NMDARs at synapses[55], and those lacking EphB1–3 show a reduction in the size of the postsynaptic density in the hippocampus, in addition to defective spine formation[44]. These abnormalities are due to the absence of EphB intracellular signalling, as neurons from animals expressing only the extracellular domain of EphB2 seem to be similar to neurons lacking EphB1–3 (REF. 44). Interestingly, *EphB1*$^{-/-}$/*EphB2*$^{-/-}$/*EphB3*$^{-/-}$ mice are viable and able to breed, suggesting that EphBs control the formation of only a subset of synapses. Indeed, there are ~40% fewer synapses in the cortex of early postnatal mice, and postsynaptic specializations are specifically lost on dendritic protrusions without affecting those on the dendritic shaft[50]. These defects can be rescued by the expression of *EphB2* in individual neurons in cultured brain slices from EphB1–3-null mice, indicating that EphB2 functions cell-autonomously to control the formation and localization of a certain type of excitatory postsynaptic specialization[50]. It remains to be determined whether the apparent loss of a specific subset of synaptic inputs in neurons lacking EphBs reflects a role for EphB at particular classes of excitatory synapses, or is due to a more general defect in the competence of neurons to make synapses in the absence of EphB.

Ig superfamily members

The Ig family of cell adhesion molecules contain variable numbers of globular extracellular cysteine-looped domains[56]. A number of Ig superfamily proteins have been shown to have important functions during synapse development, ranging from instructive roles in the organization of pre or postsynaptic specializations to roles in controlling target selection (FIG. 3).

A member of the Ig superfamily, synaptic cell adhesion molecule (SynCAM) was identified in a search for proteins with an extracellular Ig domain and an intracellular PDZ binding domain[57] — two characteristics that might confer cell adhesion and synaptogenic properties to a molecule. In mammals, there are at least four SynCAM family members with a number of potential splice variants, but only the role of SynCAM1 has been described. SynCAM1 mediates cell adhesion in a calcium-independ-ent manner and can regulate the number of presynaptic specializations that form on neurons[57]. Although the *in vivo* phenotype resulting from SynCAM1 loss-of-function has not yet been reported, homophilic or heterophilic SynCAM family member interactions provide an attractive mechanism for the alignment of pre- and postsynaptic terminals early in development.

A novel family consisting of two synaptic adhesion-like molecules (SALMs) was recently identified by two groups using the PDZ domains of PSD-95 or synapse-associated protein 97 (SAP97) as bait in a yeast two-hybrid screen[58,59]. Like SynCAM1, SALMs contain an extracellular Ig domain and both SALM1 and SALM2 seem to have important functions at synapses. Overexpression of SALM1 promotes neurite outgrowth in young cultured neurons and co-clusters with both PSD-95 and NMDARs in older neurons through PDZ binding domain interactions[58]. Similar to EphBs, the extracellular domain of SALM1 appears to

interact with the NR1 subunit of the NMDAR[58]. SALM2 seems to have a particular role in synapse maturation *in vitro*. Using a combination of overexpression, siRNA knockdown and bead assays, SALM2 was shown to regulate the formation of PSD-95-containing synaptic sites and dendritic spines, as well as the clustering of AMPARs and, to a lesser degree, NMDARs[59]. Knockdown of SALM2 causes a decrease in the frequency of excitatory but not inhibitory miniature synaptic currents, suggesting that SALM2 can act postsynaptically to regulate the formation of excitatory synaptic sites. Unlike some other synaptic organizing molecules, SALM2 expressed in non-neuronal cells fails to induce the formation of presynaptic specializations at contact points in co-cultured neurons[59].

Another adhesion molecule identified in a yeast two-hybrid screen using the PDZ-binding domain of PSD-95 as bait was netrin G2 ligand (NGL2)[60]. NGL2 localizes to the postsynaptic side of excitatory synapses, promotes the formation of dendritic spines, and induces clustering of PSD-95 and NMDARs, though not AMPARs. NGL2 expressed in non-neuronal cells triggers presynaptic differentiation of contacting axons in a co-culture assay, and knockdown of NGL2 results in the loss of only excitatory synapses. Further work will be necessary to understand the specific role for SALM family members and NGL2 in synapse development, as well as the relevance of SALM and NGL2 signalling *in vivo*. It is noteworthy that both of these molecules, along with SynCAM1 and neuroligins, share the same PDZ binding motif and associate with PSD-95, highlighting the significance of interactions with this scaffolding protein in the organization of certain synapses.

Neural cell adhesion molecule (NCAM) is a single-pass transmembrane protein with multiple Ig domains that has numerous roles during development, particularly during axon guidance. A potential function for NCAM in synapse formation was suggested by its localization at synapses[61] and by results of experiments using co-cultures of $Ncam^{-/-}$ and $Ncam^{+/+}$ neurons. However, homotypic $Ncam^{-/-}$ cultures do not show decreased synapse numbers compared with wild-type cultures, indicating that although it is sufficient to promote synapse formation in a ‘choice situation’ between NCAM-positive and NCAM-negative neurons, NCAM itself is not likely to be necessary for synaptogenesis[62,63].

Several other Ig-containing proteins seem to be particularly important for determining the specificity of neuronal connections. Many of these, such as sidekicks, synaptogenesis abnormal 1 (SYG1) and SYG2, have been described in non-mammalian systems[64–66]. Neurofascin, an L1 family Ig cell adhesion molecule, has been implicated in directing the subcellular organization of GABA- containing synapses in the mouse cerebellum. The specific subcellular domain of Purkinje neurons onto which basket cells synapse is controlled by a subcellular gradient of neurofascin; in the absence of this gradient, basket axons follow neurofascin to ectopic locations[67]. So, neurofascin seems to direct synapse formation to particular locations on a target cell and reflects the role that this family of proteins might have in establishing synapse specificity.

Cadherins

Cadherins are classical adhesion molecules that form calcium-dependent homophilic bonds at many intercellular junctions[68–70]. Neuronal (N)-cadherins are found in both pre- and postsynaptic terminals[71,72]. The intracellular domain of N-cadherin is linked to the

cytoskeleton through interactions with cytoplasmic catenin molecules (αN-, β- and p120 catenins)[73], and cadherins are composed extracellularly of five ecto-domains that mediate calcium-dependent binding[74]. Cadherins form *cis*-dimers that then associate with other *cis*-dimers across the synapse to create strong adhesion complexes[75].

Evidence indicates that N-cadherins can regulate synapse development (FIG. 3). N-cadherins accumulate at sites of axon–dendrite contact prior to differentiation of either terminal, and become restricted to discrete clusters surrounding the active zone in more mature neurons[76,77]. Overexpression of a dominant-negative N-cadherin in neuronal cultures causes a perturbation of presynaptic marker protein clusters and synaptic vesicle recycling, as well as decreased clusters of PSD-95 (REF. 76); N-cadherin also associates with AMPARs via β-catenin and can regulate AMPAR trafficking[78]. The N-cadherin–β-catenin interaction is further involved in the maturation and stabilization of synaptic specializations. Enhancing β-catenin's association with N-cadherin in dendritic spines results in an increased size of postsynaptic PSD-95 clusters and presynaptic vesicle clusters, and the increased frequency of spontaneous excitatory events[79]. N-cadherin does not trigger presynaptic development in a reduced co-culture system[80], indicating that cadherins are not an inductive synaptogenic signal.

N-cadherin signals through Rho-family GTPases, via αN-, β- and p120 catenins, to control dendritic spine morphology and, in particular, spine motility[76,79,81]. Dominant-negative N-cadherin results in a shift from mushroom-shaped spines to longer filopodia-like protrusions[76], and time-lapse studies have revealed that αN-catenin has a particular role in regulating the motility of dendritic protrusions. Neurons lacking αN-catenin show increased spine motility, whereas overexpression of αN-catenin increases spine density and reduces the turnover of dendritic spines[82]. These effects require both the carboxyl terminus (which binds actin) and amino terminus (which binds β-catenin) domains of αN-catenin[82]. So, the cadherin/β-catenin/αN-catenin system seems to be involved in the stabilization, and possibly expansion, of synaptic contacts.

A recent study has found that the *in vivo* loss of p120 catenin results in abnormal spine and synapse formation in the developing hippocampus[81] (TABLE 4). Interestingly, the reduced spine density is due to aberrant Rho family GTPase signalling independent of p120 catenin's role in regulating cadherin function and expression levels; how-ever, p120 catenin does function in the cadherin path-way to control spine maturation[81]. So, some functions of p120 catenin depend on cadherin-mediated adhesion, whereas others result from the activation of traditional intracellular signalling cascades. How these different functions are coordinated remains to be determined.

Finally, one study indicates that β-catenin might have a specific role in regulating presynaptic maturation. Synaptic terminals of mice lacking β-catenin have a smaller reserve pool of vesicles with no change in the number of docked vesicles[83] (TABLE 4). There is an increase in total synapse number in these animals, which the authors suggest is a homeostatic response to decreased synaptic efficacy[83]. Mutations in β-catenin domains important for the recruitment of αN-catenin and the association of cadherin with the actin cytoskeleton do not disrupt this activity, whereas mutation of the β-catenin PDZ binding

domain does disrupt presynaptic organization. Presynaptic β-catenin therefore functions primarily to link cadherins to PDZ domain proteins[83]. In order to understand what exact function cadherins have in presynaptic development, it will be important to resolve differences between *in vivo* results and *in vitro* evidence demonstrating the inability of cadherins to trigger presynaptic differentiation[80]. Regardless, together these data indicate that N-cadherin and catenin proteins are involved in the maturation of synaptic contacts through the control of synaptic protein clustering and dendritic morphology.

Synaptic receptor modulation and plasticity

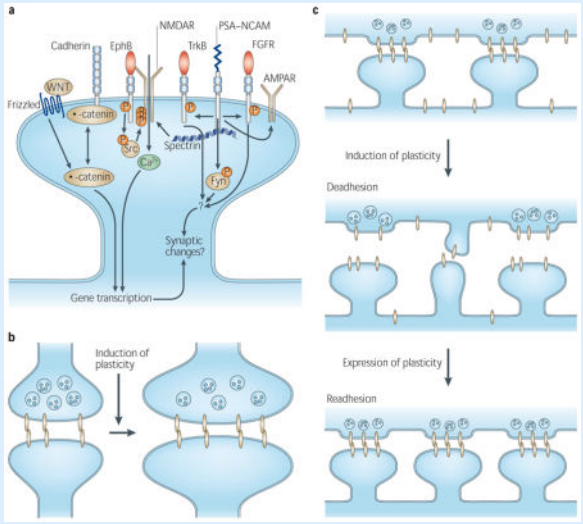
In the mature nervous system, synapses are highly organized structures, the basal properties of which are fine-tuned for reliable synaptic transmission. Synapses also undergo dynamic regulation and modulation, processes that are fundamental for adaptive nervous system functions. For example, long-term potentiation (LTP) and long-term depression (LTD) in the mammalian CNS are well-established experimental models of activity-dependent synaptic plasticity, which is thought to underlie learning and memory functions. Both basal synaptic transmission and synaptic plasticity rely on the coordinated activity of multiple synaptic components on both sides of the cleft. Therefore, molecules such as SAMs that interact with intracellular, extracellular and trans-synaptic machinery are well-suited to regulate synaptic transmission and plasticity. In addition, the mechanisms of synaptic transmission and plasticity vary between synapses[84–86] and might depend on the particular SAMs at those sites, similar to their proposed role in specifying synapses during development. However, the multifunctional nature of these molecules has made it difficult to determine exactly how they regulate synaptic function (BOX 1). Other cell adhesion molecules, such as integrins, have been implicated in synaptic function[87] but are generally thought to mediate cell–substrate interactions and are therefore not discussed here.

Box 1

Trans-synaptic adhesion molecules in synaptic plasticity

Several mechanisms could mediate the regulation of synaptic plasticity by cell adhesion molecules. These are not necessarily mutually exclusive, and a single molecule could regulate synaptic strength using several different mechanisms. Trans-synaptic interactions might initiate intracellular signalling cascades that lead to the induction or expression of synaptic plasticity. Synaptic adhesion molecules are known to interact with a number of intracellular signalling molecules that influence synaptic strength. For example, postsynaptic EphBs bind and modulate the activity of NMDARs (N-methyl-D-aspartate receptors)[43,90,] interact with AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors)[50], and induce kinase-dependent changes in dendritic spine morphology[44,49,51]. An example of this model is the role of EphB–ephrin-B interactions in mossy fibre long-term potentiation (LTP)[46,93] (see figure, part **a**). In addition, polysialic acid (PSA)–neural cell adhesion molecule (NCAM) interacts with tyrosine receptor kinase B (TrkB)[102], spectrin[119], fibroblast growth factor receptor (FGFR)[122] and Fyn[123] signalling as well as directly modulating AMPAR activity[117]. Structural changes that occur during synaptic plasticity such as changes in synaptic size or number can be stabilized by synaptic adhesion molecules (see figure, part **b**). Recent

evidence has demonstrated that activity can regulate dendritic spine head size within minutes of stimulation, and that this plasticity might underlie early forms of synaptic plasticity[141]. In addition, LTP is accompanied by an increase in synaptically localized NCAM[112] and cadherin[126], indicating that cell–cell adhesion molecules might stabilize these types of structural plasticity. Synaptic adhesion might decrease during plasticity to permit structural changes (see figure, part **c**). Experiments with both NCAM and cadherins indicate that the downregulation of adhesion mediated by these molecules might be important during early LTP[101,115,127].



Neurexins and neuroligins

Although much less is known about the function of neurexins and neuroligins at mature synapses relative to their developmental roles, results from knockout animals have begun to show that these molecules are important for mature synaptic transmission (TABLE 2). A role for α-neurexins in regulating presynaptic calcium channel function has been elucidated in knockout mice with targeted deletions of one, two, or all three α-neurexin isoforms[36]. Loss of α-neurexins resulted in decreases in whole-cell calcium currents without alterations in N- and P/Q-type calcium ion channel expression, which leads to a decrease in the frequency of both AMPAR and $GABA_A$ receptor miniature synaptic potentials[36]. The specificity of this phenotype to α-neurexin deletion was confirmed by rescue experiments in which calcium channel function was rescued by the transgenic expression of 1α-neurexin but not 1β-neurexin[36,88]. Disruptions in synaptic function have also been seen in mice lacking all three neuroligin isoforms, which have reduced GABA-mediated and glutamatergic synaptic transmission but normal synapse number in brainstem nuclei[32]. It is not known how the neuroligin family regulates synaptic function at these sites or how the neuroligin knockout phenotype is related to that of α-neurexin. Moreover, it is not known whether this family of molecules is important for activity-dependent changes in synaptic strength. However, taken together, these data demonstrate a role for the α-neurexin and neuroligin families in regulating basal synaptic properties.

Ephs and ephrins

Ephs and ephrins are expressed in the adult nervous system[39,42,89], where they are able to regulate glutamate receptor function and are involved in several forms of hippocampal synaptic plasticity. EphBs directly interact with NMDARs extracellularly[43] (see synaptogenesis section above), but EphBs also interact with glutamate receptors intracellularly to regulate receptor function and localization. Activation of EphBs potentiates NMDAR-dependent calcium flux through an EphB kinase domain-dependent phosphorylation of three specific tyrosine residues of the NR2B subunit; this phosphorylation results in increased cyclic AMP response element-binding protein (CREB) phosphorylation and calcium-dependent gene expression[90]. EphBs also regulate the localization of AMPARs. Activation of EphBs in cultured neurons leads to a PDZ binding domain-dependent increase in surface retention of AMPARs[50]. EphB kinase activity can also affect AMPAR membrane trafficking through cell-wide effects on endocytosis[91]. Although the ability of EphBs to interact with and modulate the function of glutamate receptors is intriguing, the direct impact of these interactions on synaptic transmission remains to be explored.

The role of Ephs and ephrins in synaptic plasticity is best understood at the mossy fibre–CA3 synapse in the hippocampus, where LTP is NMDAR-independent and is expressed as an increase in presynaptic neurotransmitter release probability[92]. An elegant set of experiments demonstrated that mossy fibre LTP involves a trans-synaptic signal from postsynaptic EphBs to presynaptic ephrin-Bs[46,93] (BOX 1). Mossy fibre LTP was inhibited by injecting competitive peptides into the postsynaptic cell that disrupt EphB–PDZ domain interactions[46], indicating that induction occurs at least in part postsynaptically and is dependent on EphB–PDZ binding domain interactions. Mossy fibre LTP was also inhibited by blocking extracellular EphB–ephrin-B interactions, confirming that a trans-synaptic EphB–ephrin-B signal is required for the presynaptic changes that underlie potentiation[46]. These effects are mediated by intracellular ephrin-B signalling; transgenic mice in which the intracellular domain of ephrin-B3 is replaced by lacZ, disrupting ephrin-B intracellular signalling while maintaining the ability of ephrin-B3 to interact with EphBs extracellularly, also have reduced mossy fibre LTP[93]. Taken together, these results indicate that mossy fibre LTP requires EphB–PDZ binding domain interactions in the postsynaptic cell followed by a trans-synaptic EphB–ephrin-B signal that results in presynaptic changes dependent on intracellular ephrin-B signalling.

Ephs have also been implicated in NMDAR-dependent synaptic plasticity at Schaeffer collateral–CA1 synapses in the hippocampus, although the mechanism of action is less clear here than at the mossy fibre synapse. LTP in this region is divided into an early form (E-LTP), which is thought to result from the insertion of AMPARs into the postsynaptic membrane combined with direct AMPAR modulation; and a late form (L-LTP), which relies on the activation of protein synthesis-dependent pathways[94]. The CA1 synapse also displays NMDAR-dependent LTD in response to low frequency stimulation. Two independent reports showed that mice lacking EphB2 have normal basal AMPA-mediated currents but reduced CA1 L-LTP[45,55] (TABLE 3). Only one of these studies, however, observed a deficit in E-LTP[55]. In addition, mice lacking EphB2 exhibit deficits in CA1 LTD and spatial

memory, as demonstrated in the Morris water maze[45]. These data showed that loss of EphB2 leads to defects in hippocampal area CA1 synaptic plasticity as well as in learning and memory functions.

The multiple links between EphBs and NMDARs suggest a potential model to explain how EphBs might regulate synaptic plasticity in CA1 through their ability to interact and modulate the function of NMDARs. Mice in which the intracellular domain of EphB2 has been replaced by lacZ have wild-type levels of LTP, demonstrating that the EphB2 extracellular domain is sufficient to rescue the loss–of-function phenotype. In addition, EphB2 nulls have fewer synaptically localized NMDARs without a change in total NMDAR expression levels[55], supporting the conclusion that EphB2 regulates plasticity in the CA1 region by organizing NMDARs at synapses through extracellular domain interactions (BOX 1). However, because EphB2/lacZ animals retain juxtamembrane tyrosines that could potentially be phosphorylated by other EphB receptor subtypes and then recruit intracellular signalling components, a potential role for intracellular EphB signalling in CA1 LTP has not been formally eliminated. Ideally, to understand the role of EphBs in synaptic plasticity, experiments would be conducted in which EphB signalling could be transiently disrupted, or the interactions between EphBs and NMDARs disrupted without otherwise affecting the function of these proteins. In any event, EphB–NMDAR interactions are likely to be important for certain forms of synaptic plasticity, while EphB effects on AMPAR trafficking and NMDAR signalling have important functional consequences during other processes such as synaptogenesis.

A role for ephrin-Bs in CA1 plasticity has also been shown. Deficits in CA1 LTP and LTD have been reported in mice lacking ephrin-B2 and in mice with two independent, targeted disruptions of the ephrin-B3 locus[47,48]; however, these deficits were not replicated in a second study of ephrin-B3 (REF. 93) (TABLE 3). Defects in spatial memory have also been reported when ephrin-B3 is disrupted[48]. Animals expressing ephrin-B3 that lacks intracellular signalling domains have normal CA1 LTP[48], indicating that ephrin-Bs regulate CA1 LTP through their extracellular domain. Unlike the mossy fibre synapse, ephrin-Bs and EphBs are co-expressed by postsynaptic cells in area CA1 (REFS 47,48), making it unlikely that a simple trans-synaptic EphB–ephrin-B signal is involved. Although a presynaptic partner for ephrin-Bs has not been determined, one candidate is EphA4, which binds ephrin-B and is localized presynaptically in this region[47]. In summary, studies in both area CA3 and area CA1 show that Ephs and ephrins are important for hippocampal synaptic plasticity, although how these molecules interact to regulate this process is not known.

NCAM

NCAM-related molecules in non-mammalian organisms were the first adhesion molecules implicated in synaptic plasticity[95,96], and NCAM has been shown to regulate this property in mammals as well. In addition to NCAM, a number of other Ig-domain containing adhesion molecules have been implicated in synaptic plasticity including L1 (REF. 97) and syntactin[98]; however, we focus our discussion on NCAM because its role in LTP is the best understood.

Multiple lines of evidence implicate NCAM in synaptic functions. Initial studies inhibited NCAM-mediated adhesion with function-blocking antibodies or synthetic peptides and found normal basal synaptic transmission but reduced E-LTP in area CA1, together with impaired hippocampal-dependent learning[97,99,100,114,142,143]. Results from *Ncam*-knockout mice are generally, but not unanimously, consistent with this result (TABLE 2). In area CA1, E-LTP defects have been seen in constitutive *Ncam* nulls[101,102] and in a temporally and cell-type restricted *Ncam* mutant[103], but not in a third, independently generated *Ncam* null[104]. Moreover, *Ncam* nulls with LTP deficits also show hippocampus-dependent long-term memory defects[103,105]. One *Ncam*-null line (*Ncam*ff+) has changes in basal synaptic transmission and short-term plasticity, but these effects do not account for LTP changes[103]. Defects in mossy fibre–CA3 LTP have also been reported for *Ncam* nulls[106], but are probably due to NCAM's role in mossy fibre lamination patterning[103,106,107]. Although it is not known why some *Ncam* nulls have LTP defects and others do not, discrepancies could be due to differences in genetic background, LTP induction protocol or extra-cellular calcium level[103,104,108,109]. Taken together, *in vitro* and *in vivo* results are difficult to interpret but indicate that NCAM has some role in hippocampal LTP.

There are several mechanisms by which NCAM might regulate synaptic strength (BOX 1). One possibility is suggested by the finding that synaptic plasticity is regulated by post-translational addition of the carbohydrate polysialic acid (PSA) to NCAM[110]. Neuronal activity increases the expression of PSA–NCAM on the cell surface[101,111,112], and specific blockade of PSA with inhibitory antibodies or enzymatic removal prevents E-LTP and LTD[101,113]. Hippocampus-dependent spatial learning protocols increase the expression levels of PSA–NCAM[114], and mice that lack the polysialyltransferase that is responsible for the addition of PSA to NCAM show normal basal synaptic transmission but defects in E-LTP and LTD[115]. Functionally, PSA weakens homophilic NCAM interactions[116], indicating that PSA–NCAM might mediate a downregulation of adhesion that is a prerequisite for structural changes that underlie plasticity (BOX 1). In this model, PSA linkage acts as a switch between structural stability and plasticity; however, this model has not been verified.

PSA–NCAM might also indirectly regulate synaptic plasticity through interactions with other synaptic proteins (BOX 1). Currently, NCAM and PSA–NCAM have been shown to interact with a large number of molecules or signalling pathways that regulate aspects of LTP including AMPARs[117], NMDARs[118], brain-derived neurotrophic factor (BDNF)–tyrosine receptor kinase B (TrkB) signalling[102], the spectrin-based scaffold[119–121], the fibroblast growth factor (FGF) receptor[122] and the non-receptor tyrosine kinase Fyn[123]. In most of these cases, a direct link between the interactions and LTP has not been demonstrated. The exception is BDNF–TrkB signalling, where defects in LTP caused by enzymatic PSA removal are rescued by exogenous treatment with BDNF, indicating that PSA-dependent defects in LTP are due to decreased BDNF signalling[102]. Nevertheless, the numerous interactions between NCAM and LTP-influencing pathways show that NCAM is a multifunctional protein capable of influencing synaptic plasticity at multiple levels. This highlights the need to either structurally or functionally dissociate these interactions to fully understand how SAMs are involved in a complex process such as synaptic plasticity.

Cadherins

Cadherins are expressed in the adult nervous system and localize to synaptic sites[71,72,124–126]. Blockade of extracellular N- or E-cadherin adhesion attenuates E-LTP at Schaeffer collateral–CA1 synapses without affecting basal synaptic properties[127]. Similarly, an anti-body specific to the protocadherin arcadlin abolished tetanus-induced E-LTP, although this antibody also decreases basal excitatory transmission[128]. In both cases, antibody-mediated inhibition is calcium dependent, suggesting a model in which cadherins are only avail-able to inhibitory reagents when adhesion is disrupted by a decrease in extracellular calcium. Such disruption might occur physiologically, as rapid patterns of activity can transiently decrease extracellular calcium[127] (BOX 1). N-cadherin can also regulate presynaptic function and short-term plasticity in embryonic stem cell-derived neurons from N-cadherin-null mice co-cultured with wild-type neurons[129].

Confirming a role for cadherin in long-term synaptic plasticity with knockout animals has been complicated by the size of the cadherin gene family as well as by the crucial roles of cadherin during development and in intracellular signalling (TABLE 4). However, mice lacking a specific form of cadherin expressed in the hippocampus, cadherin 11, show increases in CA1 E-LTP without changes in basal synaptic properties[130]. Cadherin 11-deficient mice have altered responses in a startle paradigm and an elevated plus maze but not in the Morris water maze, indicating that cadherin is involved in anxiety responses but not spatial memory[130]. Mice that express a dominant-negative E-cadherin that lacks the extracellular domain in hippocampal neurons but not in glia show normal electrophysiological properties at excitatory synapses, indicating a role for glial cadherin in LTP[131]. The differences between *in vivo* results and results acquired *in vitro* using blocking reagents could reflect compensation by functionally redundant cadherins or other adhesion molecules. Recently, the problem of analysing synaptic function in embryonic lethal N-cadherin nulls has been overcome by inducing neuronal differentiation of pluripotent embryonic stem cells *in vitro* from N-cadherin-null mice[129]. This approach has demonstrated a role for N-cadherin in vesicle release and short-term plasticity, but has not been used to address long-term synaptic plasticity. Further work will be necessary to resolve the role of cadherins in hippocampal synaptic plasticity.

A number of lines of evidence indicate possible mechanisms by which cadherin family molecules might be involved in LTP (BOX 1). First, cadherins could be involved in stabilizing structural plasticity. Cadherin localization and dimerization are regulated by NMDAR activation, which could lead to activity-induced increases in cadherin-mediated adhesion[132]. In addition, pharmacological induction of L-LTP leads to protein synthesis-dependent increases in cadherin expression and the number of cadherin-positive synaptic puncta[126]. Cadherins might also regulate synaptic plasticity through interactions with catenins. Depolarization of neurons in culture leads to an accumulation of β-catenin in dendritic spines, where it modifies synaptic strength by interacting with cadherins[79]. Mice lacking the neuron-specific Δ-catenin exhibit deficits in CA1 LTP and hippocampus-dependent memory tasks[133], and loss of αN-catenin results in long-term memory defects[134]. Cadherin and β-catenin also form complexes with AMPARs and can regulate AMPAR trafficking[78], although the significance of this in synaptic plasticity has yet to be

demonstrated. Finally, as discussed above, cadherins have a well-identified role in structural plasticity and dendritic spine formation. Taken together, these studies demonstrate properties that are consistent with changes thought to be important in LTP, but further work will be required to establish direct links between these properties and synaptic plasticity.

Conclusions

Trans-synaptic interactions affect the function of synapses at multiple levels, from recruiting proteins and shaping morphology during synaptogenesis to modulating the function of synaptic channels and receptors and regulating synaptic plasticity. As we have described, a common feature of many SAMs during synapse development is their ability to interact with other synaptic proteins containing PDZ domains. In addition, a structure–function approach has yielded evidence in each case that specific aspects of synaptogenesis can be attributed to particular domains of a given SAM signal, be it alternative splicing of the neuroligin ectodomain guiding excitatory versus inhibitory synapse formation, or EphB kinase domain-dependent induction of spine formation. Whether similar structural motifs are important for the function of these proteins at the mature synapse remains to be determined. Interestingly, PDZ-binding domain interactions and PDZ domain-containing proteins have consistently been shown to have important roles in synaptic plasticity[135–140]. Although the potential use of similar domains could indicate that the formation of synaptic connections and synaptic plasticity share common mechanisms, the conserved function might also simply reflect the re-use of available properly positioned signalling systems for diverse purposes.

Individually, SAMs can regulate particular aspects of synaptic maturation and function. Whether these proteins function independently or together to specify the development of particular types or classes of synapses remains to be determined. However, it is already apparent that certain molecules act at certain types of synapses, both during development and in the mature nervous system. For example, specific neuroligins function at inhibitory synapses, whereas other neuroligin subtypes, EphBs, SALMs and NGL2 seem to act preferentially at excitatory synapses. Determining at which particular synapses, and in which brain areas, trans-synaptic signals have an essential role will be an important step towards understanding the need for such a large array of synaptically-localized cell adhesion molecules in the CNS.

Acknowledgments

We thank L. Gabel for helpful comments. The authors work is supported by a Whitehall Foundation, Edward Mallinckrodt Jr Foundation, Philadelphia Foundation, and Mental Retardation Developmental Disability Research Center grant to M.B.D., the Training Program in Developmental Biology to A.C.M. and by the Ruth L. Kirschstein National Research Service Award to M.S.K.

Glossary

Synapse formation	The initial contact of two neurons and organization of the earliest components of a young synapse, including presynaptic vesicles and release machinery, and postsynaptic NMDA receptors and PSD-95

Synaptic plasticity	The ability of certain patterns of activity to lead to increases or decreases in synaptic strength
Synaptogenesis	The entire process that leads to a fully functional synapse, including cell–cell contact, differentiation of nascent preand postsynaptic terminals, development of morphological specializations, and ultimately the organization of mature synaptic inputs
Alternative splicing	The production of different proteins from the same RNA transcript by combining splice donor and acceptor sites in different combinations
PDZ binding domain	Protein domains that typically bind specific carboxy-terminal sequences in target proteins. Many proteins contain one or more PDZ domains, which were named after the initial three members (PSD-95, *Drosophila* discs large protein and ZO-1)
Miniature synaptic current	The postsynaptic current evoked by release of a single vesicle of neurotransmitter — the quantal amplitude
Synapse maturation	Expansion and stabilization of a synapse characterized by morphological maturation into a mushroom-shaped dendritic spine, additional recruitment of synaptic proteins necessary for plasticity such as AMPA receptors, and other events leading to normal synaptic transmission
Adaptor protein	A protein that contributes to cellular function by recruiting other proteins to a complex. Such molecules often contain several protein–protein interaction domains
Yeast two-hybrid screen	System used to determine the existence of direct interactions between proteins. Two hybrid proteins are expressed together in yeast; one is fused to the GAL4 DNA-binding domain and the other is fused to the GAL4 activation domain. If the proteins interact, the resulting complex drives the expression of a reporter gene, commonly β-galactosidase
Basket cells	Inhibitory interneurons located in the molecular layer of the cerebellum. Basket cells are located close to Purkinje cells and are spread out horizontally
Active zone	A portion of the presynaptic membrane that faces the postsynaptic density across the synaptic cleft. It constitutes the site of synaptic vesicle clustering, docking and neurotransmitter release
Synaptic vesicle recycling	The process whereby synaptic vesicles release neurotransmitter, are reformed and refilled with neurotransmitter to be re-used in synaptic release

Long-term potentiation	(LTP). The prolonged strengthening of synaptic inputs, which is induced by patterned input and is thought to be involved in learning and memory formation
Long-term depression	(LTD). A persistent reduction of synaptic strength in response to weak, poorly correlated input
Miniature synaptic potentials	Synaptic potentials observed in the absence of presynaptic action potentials; they are thought to correspond to the response elicited by a single vesicle of transmitter.
Morris water maze	A task used to assess spatial memory, most commonly in rodents. Animals use an array of extra-maze cues to locate a hidden escape platform that is submerged below the water surface. Learning in this task is hippocampus-dependent
Synaptic puncta	The cluster of synaptic proteins labelled with antibodies raised against various synaptic marker proteins

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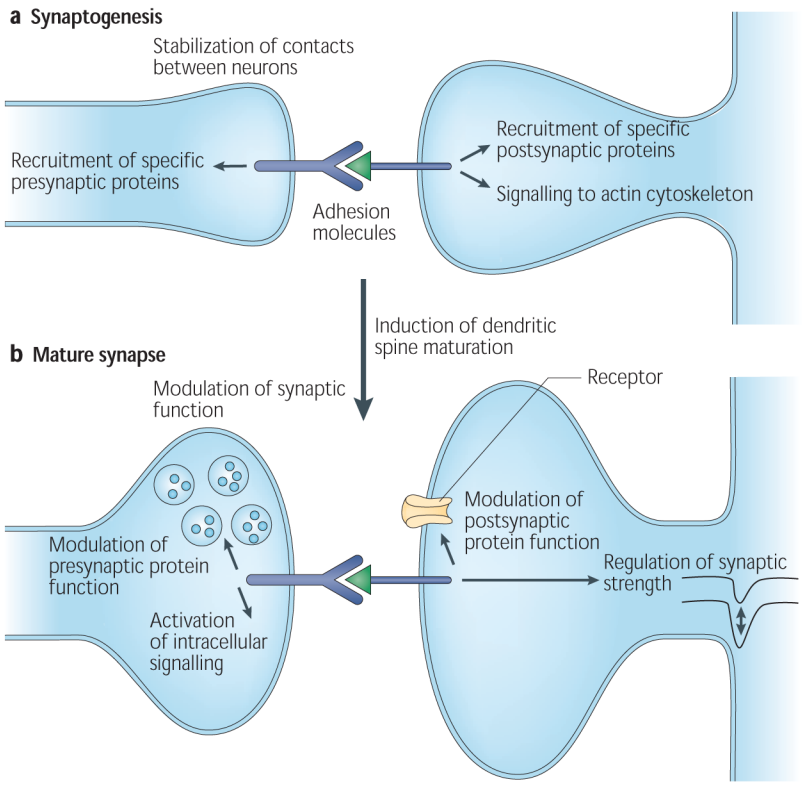

Figure 1. Synaptic adhesion molecules function throughout the life of a synapse

a | At the nascent synaptic site, synaptic adhesion molecules stabilize the initial contact between axons and dendrites. Clustering and binding of adhesion proteins can lead to the recruitment of synaptic proteins via specific cytoplasmic or extracellular domains on these molecules, including PDZ-binding domains. Interactions between adhesion molecules can also lead to the activation of intracellular signalling events that can drive synapse maturation. In particular, signalling to the actin cytoskeleton can lead to the induction of dendritic spine formation. **b** | In the mature or maturing synapse, synaptic adhesion molecules can interact with channels and other synaptic proteins to modulate their function, either by direct interaction with these proteins or through the activation of intracellular signalling events. In addition, synaptic adhesion proteins can regulate synaptic plasticity.

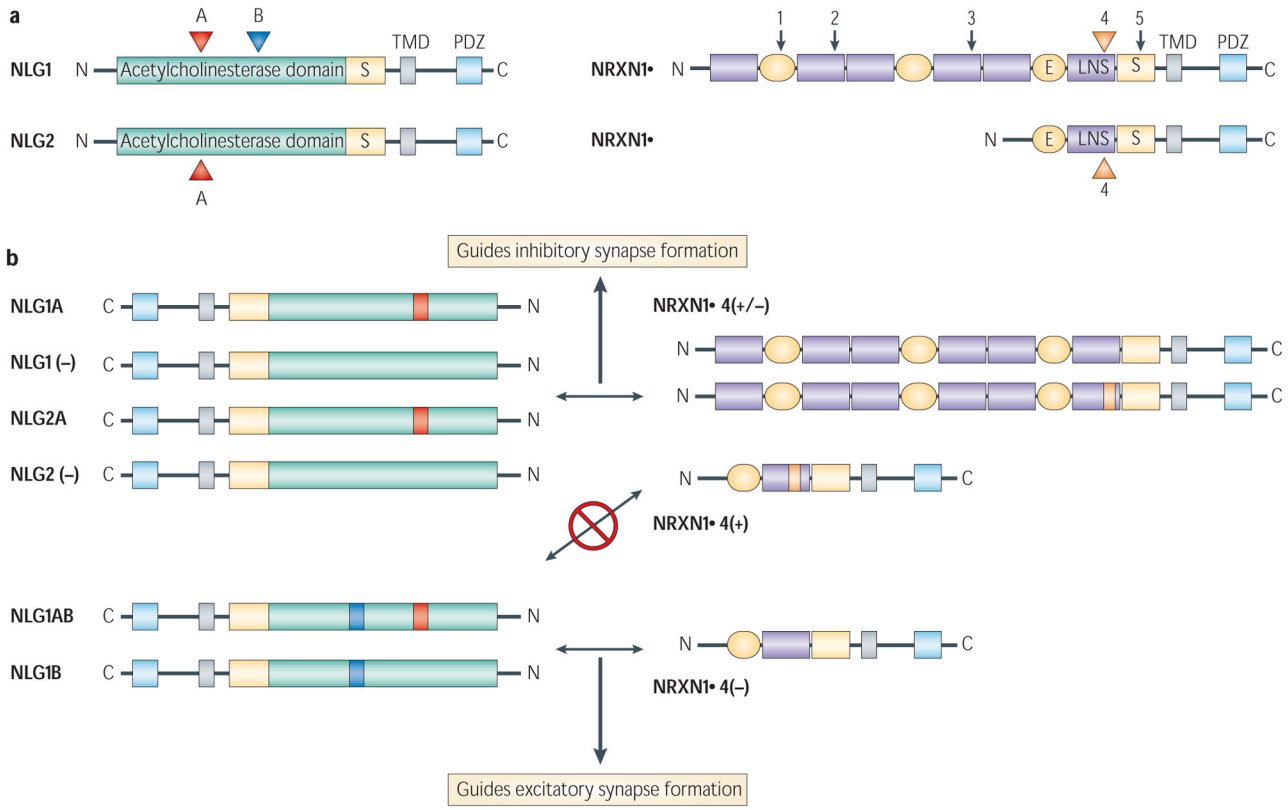

Figure 2. Neurexin–neuroligin splice code and synapse formation

Alternative splicing of presynaptic neurexins and postsynaptic neuroligins can guide the formation of GABA (γ-aminobutyric acid)-containing or glutamatergic synapses. **a** | Potential sites of inclusion of short alternative exons in neuroligins (NLG) 1 and 2, and neurexin (NRXN) 1α and 1β are shown by arrows and coloured arrowheads. We focus on splice site 4 in neurexins because the most evidence exists for the role of that site in regulating synapse development. **b** | Shows the splice code that guides interactions between neurexins and neuroligins, as well as formation of excitatory or inhibitory synapses[12,13,24]. β-neurexins containing the insert at site 4 (orange) or α-neurexins with or without that insert bind neuroligins 1 or 2 with no inserts or containing the insert at site A only (red). These interactions preferentially guide the formation of an inhibitory synaptic contact. Neuroligin 1 containing the insert in site B (blue), with or without the insert in A, is largely restricted to interactions withβ-neurexins lacking an insert at site 4. These interactions preferentially guide the formation of an excitatory synaptic contact. Although these splice variants significantly affect the interactions between neurexins and neuroligins, and formation of excitatory or inhibitory contacts, none is absolute. For example, NLG1(−), NLG1A, NLG2(−), NLG2A and NRXN1β4(−) are more promiscuous and can induce excitatory or inhibitory synapse formation in a reduced *in vitro* system. In neurons, the function of some of these variants might be restricted by their localization. C, carboxy-terminal domain (cytoplasmic); E, epidermal growth factor-like sequence; LNS, laminin-/neurexin-/sex hormone-binding globulin domain; N, amino-terminal domain (extracellular); PDZ, PDZ

binding domain; S, carbohydrate attachment site; TMD, transmembrane domain. Panel **a** modified, with permission, from REFS 12,13 © (2006) Elsevier Science.

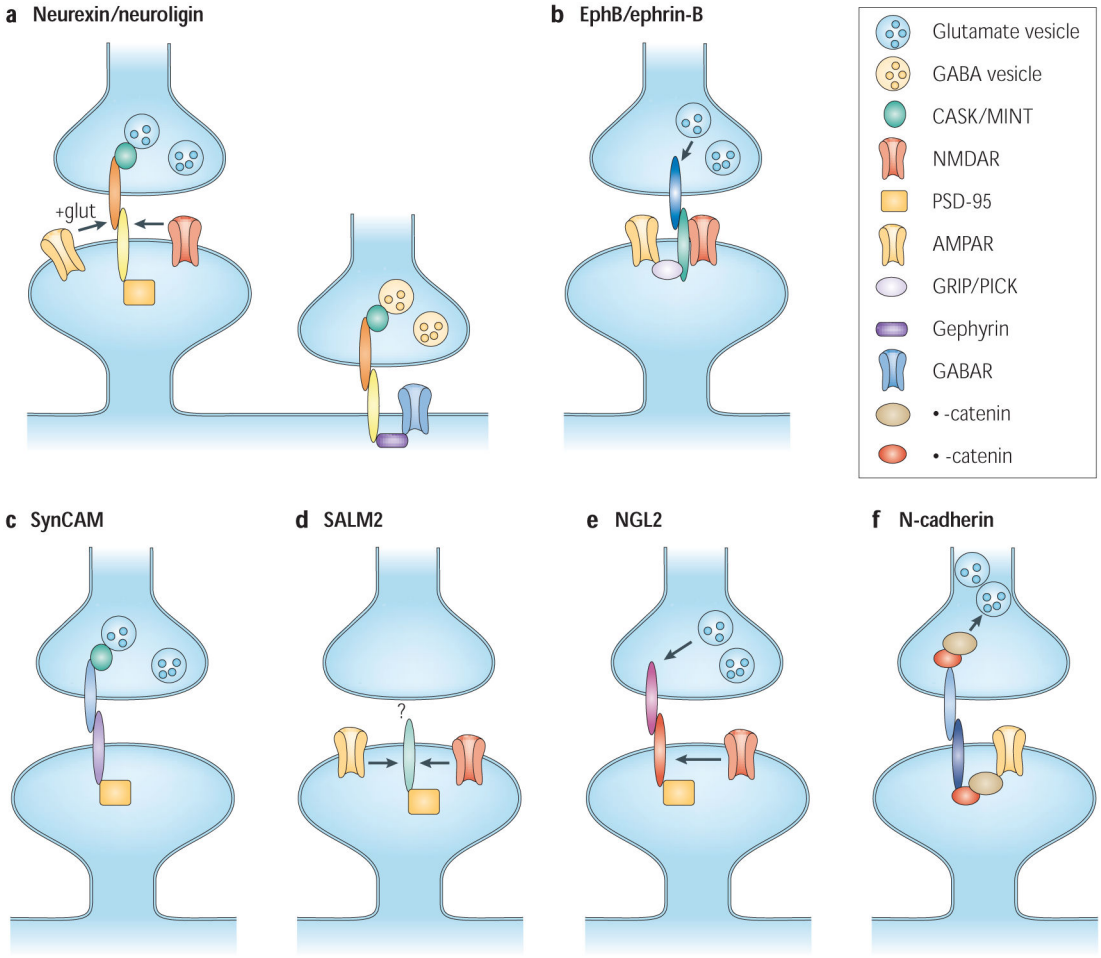

Figure 3. Trans-synaptic signalling during synaptogenesis: *in vitro* evidence

A number of trans-synaptic adhesion molecules are able to control different aspects of synapse development in neuronal cultures and heterologous cell co-cultures. **a** | Neurexins and neuroligins can induce the formation of both excitatory and inhibitory synapses.**b** | EphBs can organize both pre- and postsynaptic glutamatergic terminals through mechanisms requiring defined EphB2 protein domains, and signal to induce dendritic spine formation. **c** | Synaptic cell adhesion molecule (SynCAM) triggers presynaptic maturation but does not yet have a defined role in postsynaptic differentiation. **d** | Synaptic adhesion-like molecule 2 (SALM2) can regulate the organization of the postsynaptic terminal but not the presynaptic terminal (its presynaptic ligand is unknown). AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors), and to a lesser degree NMDARs (*N*-methyl-D-aspartate receptors), can be found at SALM2-induced synapses. **e** | Netrin G2 ligand (NGL2) can organize pre- and postsynaptic terminals of excitatory synapses. NMDARs are recruited to these sites, but not AMPARs.**f** | Finally, N-cadherin and catenins are required for the formation of normal presynaptic vesicle reserve pools and have a well-described role in the formation, development and stability of dendritic spines by signalling through Rho GTPases. Cadherins also associate with AMPARs through β-catenins. Arrows indicate that recruitment/clustering occurs, but the mechanism is unclear. '?' indicates that the

presynaptic ligand is unknown. CASK, calcium/calmodulin-dependent serine protein kinase; GABAR, γ-aminobutyric acid receptor; GRIP, glutamate receptor interacting protein; MINT, (Munc 18 interacting protein; lin-10/X11); PICK, protein interacting with C kinase; PSD-95, postsynaptic density protein-95.

Table 1

In vitro evidence for trans-synaptic signalling in synaptogenesis

SAM	Excitatory postsynaptic differentiation	Inhibitory postsynaptic differentiation	Presynaptic differentiation	Dendritic spine formation
Neurexins/neuroligins	Direct association with PSD-95 via PDZ binding domain interactions[14]; NMDARs recruited by neuroligin[17,20], but mechanism unclear; AMPARs recruited in co-culture only in the presence of glutamate[22]	Recruitment of GABAR and gephyrin in co- culture[20]; fewer inhibitory synapses with neuroligin knockdown[17]	Induced in co-culture[21]; neurexin associates with CASK/MINT[15,16,25]	Induced with overexpression of neuroligins[17]; mechanism unknown
EphBs/ephrin-Bs	Direct extracellular domain interaction of EphB2 with NMDARs[43]; EphB2 also associates with AMPARs via PDZ binding domain interactions[46,50]	ND	Induced in co-culture and requires ephrin binding domain[50]; mechanism unknown	Induced by EphBs/ephrin- Bs via multiple signalling pathways (Rho GEFs, syndecan, FAK)[44,49,51,53,54]. Fewer spines form with expression of EphB2 kinase inactive mutant[51]
SynCAM	Direct association with PSD-95 via PDZ binding domain interactions[57]	ND	Induced in co-culture[57]; SynCAM binds CASK/ MINT[57]	ND
SALM2	Direct association with PSD-95 via PDZ binding domain interactions[59]; AMPARs recruited in co-culture, but NMDARs less so[59]	ND	No induction in co- culture[59]; presynaptic ligand is unknown	Induced with overexpression[59]; mechanism unknown
NGL2	Direct association with PSD-95 via PDZ binding domain interactions[60]; NMDARs recruited in co-culture, but not AMPARs[60]	ND	Induced in co-culture and requires NGL2 extracellular domain[60]; mechanism unknown	Induced with overexpression of NGL2 via PSD-95- dependent and -independent mechanisms[60]
N-cadherin	Fewer clusters of PSD-95 with inhibition of cadherin signalling[76]; evidence for interaction with AMPARs[78]	ND	No induction in co- culture[80] (but see *in vivo* evidence in TABLE 2)	Fewer spines with N-cadherin dominant-negative[76]; α-catenin stabilizes spines[82]; p120 required for spine formation and maturation[81]

Not all *in vitro* and *in vivo* results are consistent. See TABLES 2–4 for *in vivo* studies. AMPARs, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors; CASK, calcium/calmodulin-dependent serine protein kinase; FAK, focal adhesion kinase; GABAR, γ-aminobutyric acid receptor; GEFs, guanine nucleotide exchange factors; MINT, (Munc 18 interacting protein; lin-10/X11); ND, not determined; NGL2, netrin G2 ligand; NMDARs, *N*-methyl-D-aspartate receptors; PSD-95, postsynaptic density protein-95; SALM2, synaptic adhesion-like molecule 2; SAM, synaptically localized cell adhesion molecule; SynCAM, synaptic cell adhesion molecule.

Nat Rev Neurosci. Author manuscript; available in PMC 2016 February 17.

Table 2

Neurexins, neuroligins and NCAM: *in vivo* evidence for roles in developing and mature synapses

Genotype	Description	Basal synaptic transmission	LTP	LTD	Learning and memory	Synapse formation
Nrxn1α $^{-/-}$/*2α* $^{-/-}$/*3α* $^{-/-}$	Triple knockout of three α-neurexin genes	Decreased presynaptic release secondary to impaired calcium entry[36]	ND	ND	ND	Fewer inhibitory synapses by EM[36] (see text)
Nlg1$^{-/-}$/*2*$^{-/-}$/*3*$^{-/-}$	Knockout of the 3 neuroligins most highly expressed in newborn mice; animals die of respiratory failure	Reduced sPSC frequency in PBC (inhibitory more so than excitatory)[32]	ND	ND	ND	No loss of synapse number; increased ratio of excitatory versus inhibitory synapses[32]
Ncam$^{-/-}$	*Ncam* null	Normal[106]	Decreased in CA1[101,102]; decreased in CA3[106]	ND	Spatial learning deficits[105]	Defective mossy fibre laminations[106,107]
*Ncam*ff+	Conditional knockout of NCAM in hippocampus	Increased basal synaptic transmission; decreased PPF[103] (see text)	Decreased in CA1; normal in CA3[103]	Decreased in CA1[130]	Delay in spatial memory acquisition that can be overcome by training[103]	Normal mossy fibre laminations[103]
Ncam$^{lacZ/lacZ}$	NCAM null in which the full NCAM coding sequence is replaced by lacZ	Normal[104]	Normal in CA1[104]	ND	ND	Phenotype is similar to *Ncam*$^{-/-}$ [104]
ST8SiaIV $^{-/-}$	Mice lacking one of the polysialytransferases responsible for adding PSA to NCAM	Normal[115]	Decreased in CA1; normal in CA3[115]	Decreased in CA1[115]	ND	Normal mossy fibre laminations[115]

EM, electron microscopy; ff+, floxed; LDP, long-term depression; LTP, long-term potentiation; *Ncam*, neural cell adhesion molecule; ND, not determined; *Nlg*, neuroligin; *Nrxn*, neurexin; PBC, pre-Bötzinger complex of brainstem respiratory centre; PPF, paired pulse facilitation; PSA, polysialic acid; sPSC, spontaneous postsynaptic current.

Table 3

Ephs and ephrins: *in vivo* evidence for roles in developing and mature synapses

Genotype	Description	Basal synaptic transmission	LTP	LTD	Learning and memory	Synapse formation
EphB2$^{-/-}$	EphB2 null	Normal basal synaptic transmission[45,55]; decreased synaptic NMDA currents[55]	Decreased in CA1[45,55]	Decreased in CA1[45]	Impaired spatial memory[45]	Fewer NMDARs at synapses[55]; normal CA1 synapse number by EM[55]; slightly increased CA1 synapse number by EM[45]
EphB2$^{lacZ/lacZ}$	Transgenic line in which intracellular domain of EphB2 is replaced by lacZ	Normal basal synaptic transmission[45]	Normal in CA1[45]	Normal in CA1[45]	Normal spatial memory[45]	Total synapse number in CA1 equal to wild type by EM[45]
EphB1$^{-/-}$/*2*$^{-/-}$/*3*$^{-/-}$	Mouse lacking all three neuronally expressed EphBs	ND	ND	ND	ND	Fewer dendritic spines[44,50]; reduced size of PSD[44]; fewer synapses[44,50]
EphA4$^{-/-}$	EphA4 null	Normal[45]	Decreased in CA1[45]	Decreased in CA1[45]	ND	Total synapse number in CA1 equal to wild type by EM[45]
EphA4$^{EGFP/EGFP}$	Transgenic line in which intracellular domain of EphA4 is replaced by EGFP	Normal[45]	Normal in CA1[45]	Normal in CA1[45]	ND	Total synapse number in CA1 equal to wild type by EM[45]
ephrin-B2- CaMKcre	Conditional knockout of ephrin-B2 in the postnatal forebrain	Normal[47]	Decreased in CA1[47]	Decreased in CA1[47]	ND	Total synapse number in CA1 equal to wild type by EM[47]
ephrin-B3$^{-/-}$	ephrin-B3 null	Normal[47]	Decreased in CA1[47]	Decreased in CA1[47]	ND	Total synapse number in CA1 equal to wild type by EM[47]
ephrin-B3$^{neo/neo}$	ephrin-B3 hypomorph	Normal[48,93]	Decreased in CA1[48]; normal in CA1[93]	ND	Impaired spatial memory[48]	Excitatory synapse number increased in CA1 compared to wild type, equal in CA3 by EM[48]
ephrin-B3$^{lacZ/lacZ}$	Transgenic line in which intracellular domain of ephrin-B3 is replaced by lacZ	Normal[48,93]	Decreased in CA1[48]; Normal in CA1[93]; decreased in CA3[93]	ND	Normal spatial memory[48]	Excitatory synapse number increased in CA1 compared to wild type by EM[48]

CaMK, calcium/calmodulin-dependent kinase; EGFP, enhanced green fluorescent protein; EM, electron microscopy; LTD, long-term depression; LTP, long-term potentiation; ND, not determined; NMDARs, *N*-methyl-D-aspartate receptors.

Table 4

Cadherin and related molecules: *in vivo* evidence for roles in developing and mature synapses

Genotype	Description	Basal synaptic transmission	LTP	LTD	Learning and memory	Synapse formation
Cadherin 11$^{-/-}$	Null for one cadherin family member	Normal[130]	Increased in CA1[130]	ND	Normal spatial learning[130]	Total synapse number in CA1 equal to wild type by EM[130]
PCALLΔE/Cre	Transgenic line expressing dominant-negative cadherin	Normal[131]	Normal in CA1[131]	Normal in CA1[131]	Normal spatial memory[131]	ND
β-catenin$^{-/-}$	Conditional deletion of β-catenin from hippocampal pyramidal neurons after synapse formation	Increased in β-catenin nulls, possibly as a homeostatic response[83]	ND	ND	ND	Abnormal (small) vesicle reserve pool[83]
p120 catenin$^{-/-}$	Conditional deletion of p120 catenin from dorsal forebrain	ND	ND	ND	ND	Fewer spines and synapses in hippocampus[81]
δ-catenin$^{-/-}$	δ-catenin null	Decreased PPF at CA1[133]	Increased in CA1 with 100 Hz stimulation[133]; decrease in CA1 with 10 Hz stimulation[133]	ND	Spatial learning deficits[133]; fear conditioning deficits[133]	ND
cdf/cdf	Spontaneous mutant that lacks a portion of αN-catenin	ND	ND	ND	Fear conditioning deficits[134]	ND

EM, electron microscopy; ND, not determined; PPF, paired pulse facilitation.