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Review

The neuroligin and neurexin families: from structure to function at the synapse

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Abstract. Proper brain connectivity and neuronal transmission rely on the accurate assembly of neurotransmitter receptors, cell adhesion molecules and several other scaffolding and signaling proteins at synapses. Several new exciting findings point to an important role for the neuroligin family of adhesion molecules in synapse development and function. In this review, we summarize current knowledge of the structure of neuroligins and neurexins, their potential binding partners at the synapse. We also discuss their potential involvement in several aspects of synapse development, including induction, specificity and stabilization. The implication of neuroligins in cognitive disorders such as autism and mental retardation is also discussed.

Keywords. Synapse formation; cell adhesion molecule; neuroligin; neurexin; scaffolding protein.

Introduction

Synapses are specialized sites of cell-cell contact that allow communication between neurons [1–7]. The formation of these contacts is not a random process, and during development, precise and tightly regulated mechanisms are thought to dictate the location, number and type of synapses, leading to the formation of highly reproducible synaptic networks. Several new discoveries in the past 10 years have unraveled some of the critical processes that govern central nervous system (CNS) synapse development [5, 6, 8–11]. A great deal of work suggests that cell adhesion molecules orchestrate the molecular and cellular events involved in synapse formation [7, 8, 12–20].
Synapse development is thought to involve several characteristic steps: contact initiation, recruitment of presynaptic and postsynaptic proteins, stabilization/maturation and elimination [16, 21]. Initially, a contact is made between an axon and a target postsynaptic cell. An important criterion for target recognition is specificity, since correct wiring is essential to the proper function of a neural network. To ensure appropriate connectivity, this process is controlled by several mechanisms. First, there are several cues that guide axons to a precise target field, to recognize a specific cell type, possibly through sampling potential targets [22]. An example of target specificity is the precise targeting of gamma-aminobutyric acid (GABA)-ergic inputs of basket and stellate cells onto Purkinje cell dendrites and axon initial segments, respectively [23, 24]. Cell adhesion molecules mediating the specificity of initial contacts are expected to be highly polymorphic in order to offer sufficient combinational possibilities. Molecules such as cadherins, protocadherins and sidekick are thought to be involved in target recognition [16, 21, 25].
The second step in synapse formation involves the recruitment of presynaptic and postsynaptic molecules at newly formed contacts. At this stage, mismatching is likely to be prevented by the ability of trans-synaptic cell adhesion systems to control the recruitment of the proper neurotransmitters at the presynaptic site with their specific receptors at the postsynaptic site. Potential regulators of this process include the neuroligin/neurexin adhe-

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sion complex and SynCAM, which have been shown to induce clustering of several synaptic proteins [26–29].

Following the recruitment of presynaptic and postsynaptic proteins, the neuronal network is refined, as newly formed synapses are either stabilized or eliminated. Stabilization, a process involving corresponding changes in the size and content of the pre- and postsynaptic sites, is also thought to involve adhesion molecules, whose identity, however, remains unclear. Compelling evidence indicates that fine-tuning of neuronal circuits is experience dependant, with strengthening or elimination of synaptic contacts relying on external stimuli and synaptic activity. Studies on the visual, somatosensory and motor cortex have provided insights on how sensory experience can shape the maturation of cortical circuits [30–36]. Adhesion systems important for these processes are likely to be modulated by synaptic activity, which could alter their expression or adhesive properties.

In this review we will summarize data obtained from several new exciting findings that postulate an important role for neuroligins and their binding partners in synapse development and function. Initially, neuroligins were identified as ligands for β-neurexin, and a role for them in mediating cell-cell adhesion was proposed [37]. Later, neuroligins were shown to be sufficient to induce the formation of new functional presynaptic terminals *in vitro* [28]. Since this discovery, neuroligins and neurexins have received much attention, and extensive work has been done to characterize their involvement in the development of both excitatory and inhibitory synapses. These investigations have provided new insights into a unique role for specific neuroligin family members in governing the ratio of excitatory and inhibitory synapse formation [for recent reviews see refs. 13, 38–42].

Here, we will describe the general structure of neuroligins, and highlight specific features implicated in their binding to neurexins. We will then summarize the current knowledge regarding the involvement of neuroligins in synapse development, such as synapse induction and differentiation, and will discuss their putative involvement in other aspects of synapse function. Finally, we will review new evidence implicating neuroligins in neurodevelopmental cognitive disorders, including autism and mental retardation.

Structural features of neuroligins

Members of the neuroligin family are type I transmembrane proteins, comprised of several domains, including a cleaved signal peptide, a cholinesterase-like domain, a carbohydrate attachment region, a single transmembrane domain and a short C-terminal tail containing a type I PDZ-binding motif (Fig. 1) [43]. Neuroligin proteins have been identified in humans, rodents, chicken, *Drosophila melanogaster* and *Caenorhabditis elegans* [43–48]. Three genes encoding neuroligin family members have been identified in rat and mouse, while five genes coding for neuroligins have been identified in the human genome [46, 48].

Neuroligin 1, the best characterized member of the family, was first purified from brain lysates by affinity chromatography on immobilized β-neurexin [43]. Two additional neuroligin family members, neuroligins 2 and 3, were subsequently identified and shown to bind to β-neurexin [48]. Analysis of the currently identified members of the neuroligin family shows that these proteins share 52% sequence identity. The intracellular regions (31% identity) are less conserved than the extracellular and transmembrane domains, which show 55% and 91% identity, respectively [48]. Thus, one can envisage that the divergence within C-terminal domains of neuroligins may underlie the differential localizations or functions of the various family members. The extracellular region of neuroligins is responsible for heterophilic adhesion. This region contains a domain with sequence similarity to cholinesterases, members of the α/β-hydrolase fold superfamily of enzymes [49]. Neuroligins belong to a family of molecules which contain the cholinesterase-like domain (CLD), called cholinesterase-like adhesion molecules (CLAMs), which include glutactin, neurotactin and gliotactin [50]. Unlike cholinesterases, neuroligins lack one of the residues in the catalytic triad located within the CLD, which renders them enzymatically inactive. Thus, instead of mediating enzyme/substrate interaction, the CLD is thought to participate in receptor/ligand-like interaction.

Comparison of the structure of cholinesterase family members with neuroligins has greatly helped in understanding neuroligin structure/function relationships [51]. Acetylcholinesterase has three loops stabilized by disulfide bonds forming the α/β-hydrolase fold, which allows for correct positioning of the active site triad residues in the catalytic gorge mouth. The cysteine pairs forming the two N-terminal disulfide bonds are conserved in all of the neuroligin family members [48]. In vertebrates, however, the cysteines forming the C-terminal bond are shifted, resulting in a significantly shorter third loop. In contrast, this third loop is completely absent in invertebrate neuroligins. Thus, repositioning or absence of the C-terminal disulfide-bonded loop may reflect changes in the function of neuroligins throughout evolution.

All neuroligins are subject to alternative splicing at two conserved splice sites, referred to as A and B (Fig. 1) [43, 48]. The positions of the spliced regions have been mapped to loops in the CLD [48, 52]. Although the position of the spliced sites is conserved, differences in insert sequences have been reported among neuroligin family members. Sequence analysis shows that alternatively spliced sequences of rat neuroligin 1 and 2 are distinct. By

contrast, neuroligin 3 contains two types of alternatively spliced inserts, one is similar to that present in neuroligin 1, while the other is a hybrid of sequences present in neuroligin 1 and neuroligin 2 [48]. The existence of two alternatively spliced regions allows the generation of up to four different isoforms for each neuroligin gene [43, 48, 53]. Further studies are required to assess the relative levels of expression and distribution of these alternative splice forms in the brain.

Structural features of neurexins

Functions of neuroligins at the synapse have been linked to their interaction with neurexins, a family of highly polymorphic brain-specific proteins identified through a search for receptors of the black widow spider toxin, α-latrotoxin (Fig. 1) [54]. Mammalian neurexins are the product of three genes, referred to as neurexin I, II and III. From these genes, a long mRNA encoding α-neurexin and a short mRNA encoding β-neurexin, are generated. Therefore, at least six principal neurexin isoforms can be generated: three α-neurexins (Iα, IIα, IIIα) and three β-neurexins (Iβ, IIβ, IIIβ) [55, 56]. Molecular analysis of the neurexin transcripts has identified five canonical alternative splice sites in α-neurexins, and two for β-neurexins [56]. Thus, alternative splicing of various neurexin sequences can potentially give rise to more than a thousand different neurexin transcripts [57, 58]. A similar strategy is used in olfactory epithelial cells to produce a wide variety of olfactory receptors [59]. This extensive alternative splicing represents a powerful cellular mechanism for producing a multitude of distinct cell surface proteins that can be expressed within a single cell or population of cells, offering the diversity and specificity required for processes such as receptor/ligand interaction and cell-cell recognition and adhesion.

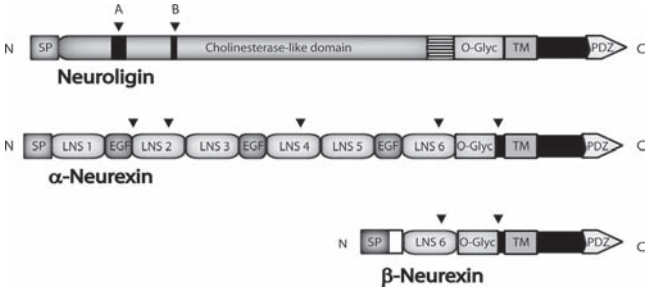

Figure 1. Structure of neuroligins and neurexins. Neuroligins are composed of a large N-terminal extracellular domain, followed by a single transmembrane region and a short cytoplasmic sequence containing a type I PDZ-recognition motif. The extracellular region of neuroligins is composed of a signal peptide (SP), followed by a cholinesterase-like domain, which mediates binding to neurexin LNS domains. The extracellular region of neuroligins contains two alternatively spliced sites (A and B), an oligomerization domain (hatched box) and a carbohydrate attachment region for O-linked glycosylation (O-Glyc). Five N-glycosylation sites and two EF-hand motifs involved in Ca^{2+} binding are also present in the extracellular domain of neuroligins (not shown). α-Neurexins are composed of an N-terminal extracellular sequence containing a signal peptide, 6 LNS domains separated by 3 EGF-like sequences, followed by an O-glycosylation region (O-Glyc), a single transmembrane domain and a short cytoplasmic region containing a type II PDZ-interaction site. β-Neurexins contain only one LNS domain, which is preceded by a β-neurexin-specific sequence (white box). Arrowheads indicate the location of the five alternative splice sites present in α-neurexins, and the two present in β-neurexins.

Notably, other proteins closely related to neurexins have been classified as the NCP family (for neurexin IV/Caspr/paranodin) [60]. This distinct subgroup of neurexins includes mammalian Caspr proteins (contactin-associated proteins, also known as paranodin) [61–64], *Drosophila* neurexin IV [65] and axotactin [66]. In contrast to mammalian neurexins, which are thought to play a role at synaptic sites, members of the NCP subgroup mediate neuron-glial and glial-glial interaction [65–67].

Both mammalian α- and β-neurexins are single transmembrane proteins, with distinct extracellular N-terminal sequences that have a receptor-like structure (Fig. 1). A domain important for the trans-synaptic signaling of neurexins is the LNS domain, named after repeated sequences present in laminin A, neurexin and sex hormone-binding protein. The LNS domain is sometimes referred to as the G domain, in reference to a sequence repeat present in laminin A, agrin and slit, proteins implicated in cell recognition processes during development of the nervous system [54, 68]. Each LNS domain is an independently folded, ligand-binding unit, and in the context of β-neurexin has been shown to be responsible for the interaction with the CLD domain of neuroligins [29, 69]. α-Neurexin has six LNS domains, which are separated by three epidermal growth factor (EGF)-like sequences (Fig. 1). In comparison, β-neurexin contains only one LNS unit, which is preceded by a β-neurexin-specific sequence resulting from the N-terminal truncation of a long atypical signal peptide [54, 55]. In both forms of neurexin, the last LNS unit is followed by a carbohydrate attachment sequence, a single transmembrane region and a short cytoplasmic tail (Fig. 1). Notably, α- and β-neurexin C-terminal regions are conserved and contain a type II PDZ-recognition motif.

Molecular determinants of neuroligin/neurexin interaction

The molecular nature of the heterophilic interaction between neuroligins and neurexins has been characterized by a combination of genetic, biochemical and biophysical approaches. These investigations have revealed that the neuroligin/neurexin interaction is regulated by a variety of cellular and molecular mechanisms, including alternative splicing, calcium binding, glycosylation and oligomerization.

Until recently, it was thought that neuroligins could only bind to β-neurexins lacking an insert at the alternatively spliced site 4 [43, 48]. However, a recent study reported that neuroligins can bind to both α- and β-neurexins, and this process depends on alternative splicing of neuroligins at splice site B [53]. This new finding opens a new window of possibilities, where differential combinations of neuroligin and neurexin isoforms and splice variants may interact at contact sites to activate distinct signaling codes that specify particular synaptic properties. *In situ* hybridization studies revealed differential but overlapping distribution of the six principal neurexin splice variants in various brain regions and classes of neuron [56]. Further studies, however, are needed to determine whether temporal and spatial distribution of various splice forms of neurexins and neuroligins regulate the binding of specific isoforms of these proteins and in turn their adhesive properties.

Neuroligin/β-neurexin binding has been shown to be calcium (Ca^{2+})-dependent [43]. Analysis of Ca^{2+} binding to the extracellular domain of recombinant neurexin 1β and neuroligin 1 revealed that neuroligin 1, but not neurexin 1β, binds Ca^{2+} [70]. Upon incubation with Ca^{2+}, no observable structural changes were detected in β-neurexin, providing further evidence that β-neurexin does not directly bind Ca^{2+} [70]. The presence of two putative EF-hand motifs suggests that neuroligins bind directly to Ca^{2+} [71]. However, whether calcium binding is critical for regulating the function of these molecules *in vivo* remains unclear.

Glycosylation provides another level of control of neuroligin/β-neurexin interaction. Neuroligin 1 contains five potential N-glycosylation sites, as well as a Ser-Thr-rich domain, proximal to the transmembrane region, which contains several candidate sites for O-linked glycosylation (Fig. 1) [43]. Treatment with glycohydrolase confirmed that neuroligin 1 is modified by both N- and O-linked sugars [43]. In comparison, β-neurexin is highly O-glycosylated but contains only one site for N-glycosylation [55]. *In vitro* studies revealed that blocking neuroligin 1 N-glycosylation increases its binding capacity for neurexin 1β [72]. Conversely, deglycosylation of the single N-glycosylation site of neurexin 1β does not affect its binding affinity to neuroligin 1. Further analysis revealed that N-glycosylation at Asn 303 located in the second splice site (site B) of neuroligin is responsible for hindering binding to neurexin 1β. Thus, alternative splicing and protein glycosylation are two key processes involved in regulating the affinity of neuroligins for binding to β-neurexins.

Protein oligomerization also controls the binding of neuroligins to neurexins. Studies on acetylcholinesterase showed that the CLD is involved in protein dimerization [73]. In neuroligin 1, mutations of residues in the CLD domain critical for protein oligomerization resulted in loss of neuroligin binding to β-neurexin [72, 74].

Spatial and temporal expression profile of neuroligins in the CNS

In situ hybridization analysis in adult rat tissue has uncovered the presence of all three neuroligin transcripts in the brain [43, 48]. A subsequent study showed that neuroligin 1 expression is restricted to CNS neurons and is localized specifically at the postsynaptic membranes of excitatory synapses [75]. Notably, neuroligin 2 is also localized at CNS synapses, but is mainly found concentrated at inhibitory synapses [29, 76]. However, analysis of mice and human cDNA databases suggests that neuroligin 2 is also expressed in tissues such as pancreas, lung, endothelia, uterus and colon. Neuroligin 3 has also been found in the brain, but some evidence suggests its expression is not restricted to neurons. In developing mice and rats, neuroligin 3 is expressed by a variety of glial cells, including immature astrocytes, Schwann cells, sattelite glia and olfactory ensheating glia [45]. In humans, three isoforms of neuroligin 3 are generated, each variant differentially expressed in brain, heart, skeletal muscle, placenta and pancreas. [77]. The neuroligin 4 gene has only been identified in humans so far, and its product is detected in diverse tissues, including heart, liver, skeletal muscle and pancreas, and at low levels in the brain [46]. The existence of a fifth neuroligin gene has also been reported [46, 78]. Neuroligin 5, also referred as neuroligin 4Y because of its localization on the Y chromosome, differs from neuroligin 4 (X linked) by only 19 amino acids [46].

Evidence suggests that the expression of neuroligins may differ between species. For example, neuroligin 3 is mainly detected in rat brain [48], whereas human neuroligin 3 and 4 are also detected in a number of peripheral tissues [46, 77, 79]. The broad tissue distribution of neuroligins raises an important question. What is the function of neuroligins outside the CNS? It is tempting to speculate that neuroligins are localized at structures involved in cell adhesion and communication, such as desmosomes, tight, adherens and gap junctions. Similar to their proposed role in neurons, neuroligin may be involved in the establishment and organization/stabilization of cell-cell contact sites in other cell types. Considering the expression of neurexins is restricted to the brain [54], additional studies are required to explore whether neuroligins have other ligand partners expressed outside the CNS.

During development, neuroligin 1 is expressed at low levels before birth, however, its expression increases between postnatal days 1–8 and remains relatively high at later stages of development and adulthood [75]. The increase of neuroligin 1 expression during early postnatal development coincides with the increased level of expression of other synaptic proteins such as the postsynaptic density protein-95 (PSD-95), in a period that correlates

with active synaptogenesis [75, 80]. In contrast with neuroligins, neurexin transcripts are detected as early as embryonic day 10 (E10), suggesting involvement in some developmental processes unrelated to synaptogenesis [81]. Thus, it will be important to determine whether differences in the expression of these molecules functionally reflect different modes of action in development and synapse signaling.

Binding partners of neuroligins and neurexins

In vitro, all neuroligin members interact through their conserved C-terminal PDZ recognition motif with PSD-95 (Fig. 2) [82]. Studies have shown that neuroligins interact specifically with the third PDZ domain of PSD-95 [82, 83]. PSD-95 is the prototypical member of the membrane-associated guanylate kinase (MAGUK) family and is comprised of three PDZ domains followed by a src homology domain (SH3) and a guanylate kinase domain. As a multidomain protein, PSD-95 has the ability to nucleate assembly of supramolecular protein complexes at the postsynaptic membrane [2]. For example, the first two PDZ domains bind to N-methyl-D-asparate-type (NMDA) glutamate receptor subunits and voltage-gated potassium channels, allowing PSD-95 to act as a scaffold linking these receptors and channels to other components of the postsynaptic machinery [84, 85]. Interestingly, overexpression of PSD-95 in neuronal cells induces clustering of other scaffolding proteins including guanylate kinase domain-associated protein (GKAP) and adhesion molecules such as neuroligins [86, 87]. PSD-95 overexpression also results in enhanced clustering and activity of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type glutamate receptors, enhanced maturation of presynaptic terminals and increased spine size [88]. Thus, coupling of neuroligins to PSD-95 may coordinate the recruitment and assembly of several postsynaptic partners, including neurotransmitter receptors, signaling molecules and the actin cytoskeleton, which are key components regulating synaptic function and structure (Fig. 2) [2]. Consistent with its role in assembly of a large protein complex of postsynaptic proteins at the PSD, knock down of PSD-95 results in reduced clustering of AMPA receptors, GKAP and Shank, and an overall decrease in the number of excitatory contacts [86, 87, 89]. However, in contrast with the striking effects on synapse maturation observed *in vitro*, PSD-95 mutant animals display normal excitatory synaptic transmission and synapse morphology [90]. Whether the observed differences are due to functional redundancy of other PDZ proteins present at the synapse remains to be determined.

In mature neurons, coupling of neuroligins to PSD-95 may regulate recruitment of other signaling molecules

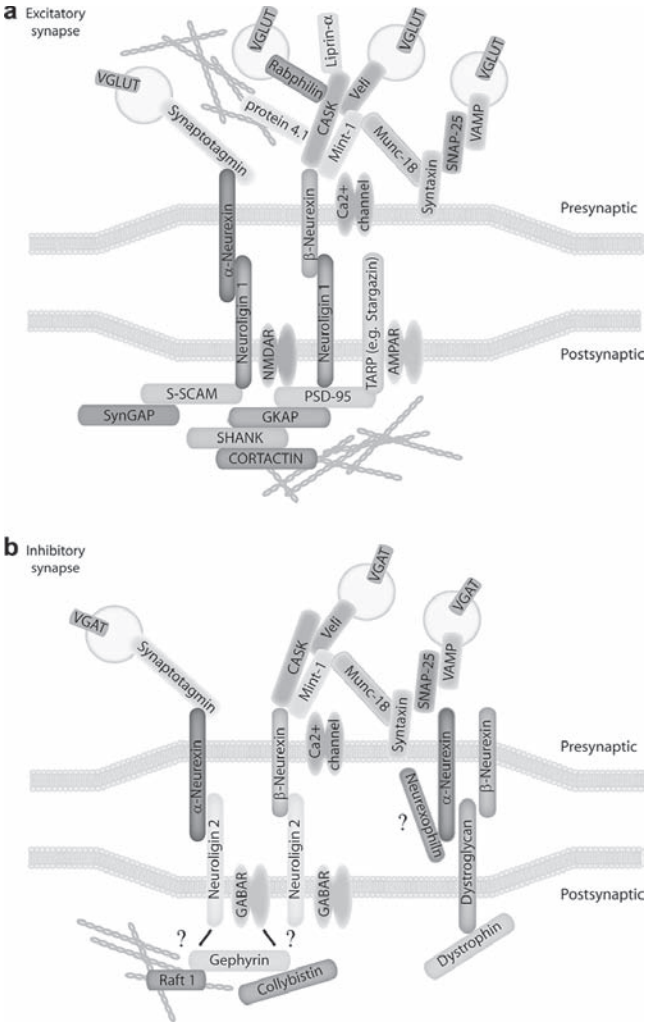

Figure 2. Protein complexes associated with neuroligins and neurexins at excitatory and inhibitory synapses. A subset of known binding partners of neuroligins and neurexins potentially involved in synapse organization are shown. Endogenous neuroligin 1 is almost exclusively located at the postsynaptic membrane of excitatory synapses (*a*), where it interacts with PDZ proteins such as PSD-95 and S-SCAM. These interactions allow coupling of neuroligin 1 to other proteins such as members of the TARP family (e.g. Stargazin), GKAP, Shank, Cortactin, SynGAP, and glutamate receptors. Neuroligin 2 is mainly detected at inhibitory synapses (*b*). No known binding partners that associate with neuroligin 2 at inhibitory contacts have been identified. However, potential regulators of neuroligin 2 localization include gephyrin, which is coupled to molecules such as Raft1 and collybistin at inhibitory contacts. At presynaptic terminals, both α- and β-neurexins can directly interact with a complex containing CASK/Veli/Mint-1 which allows recruitment of the presynaptic vesicle machinery involved in exocytosis, including presynaptic Ca^{2+} channels, Munc-18, syntaxin, liprin α and rabphilin 3a. Neurexins can also directly bind to synaptotagmins. Moreover, coupling of neurexins to the actin cytoskeleton can be mediated via interaction of CASK with protein 4.1. Whether all of the identified presynaptic partners are present at both excitatory and inhibitory terminals is unknown. In postsynaptic inhibitory synapses, both α- and β-neurexin can interact with dystroglycan, a protein that associates with dystrophin to form a complex that is selectively located at a subset of inhibitory synapses. Other potential partners of α-neurexins include neurexophilins.

that modulate synaptic transmission. For example, PSD-95 has been shown to recruit protein kinase A (PKA) to glutamate receptor complexes through interaction with AKAP79/150 [91, 92]. This association is required for the regulation of AMPA receptor activity and insertion at the synapse through PKA-mediated protein phosphorylation. PSD-95 is also indirectly coupled to AMPA-type glutamate receptor subunits, through interaction with transmembrane AMPA receptor regulatory protein (TARP) family members (e.g. Stargazin), and this protein complex has been shown to be important for modulating AMPA receptor trafficking, channel conductivity and synaptic plasticity [93–98]. Thus, it is tempting to speculate that cross-talk between PSD-95 and the neuroligin/neurexin protein complex may modulate synaptic strength by controlling retention and/or function of many associated proteins at the synapse. Moreover, trans-synaptic signaling through these proteins may be critical for coordinating changes that occur at either side of the synapse.

Experiments with the yeast two-hybrid system showed that neuroligin 1 not only binds to the PDZ domain of PSD-95, but also associates with several other PDZ proteins including SAP102, PSD-93, S-SCAM, Magi 1 and 3, Shank 1 and 3, Pick 1, GOPC, SPAR, Semcap 3 and PDZ-RGS 3 [83]. Whether all of these proteins influence the function of neuroligin at the synapse remains unclear. The interaction of neuroligin 1 with the first PDZ domain of S-SCAM, a scaffolding protein found at postsynaptic sites, has been further characterized in neurons [99]. In a recent study, S-SCAM but not PSD-95 was shown to induce synaptic clustering of neuroligin 1 [100]. However, these findings are in conflict with others which reported that coexpression with PSD-95 augments synaptic clustering of neuroligin 1 and this correlates with enhanced excitatory presynaptic terminal size [87]. Further studies are required to clarify the functional roles of the various binding partners of neuroligins *in vivo* and to determine how these interactions influence their trafficking, assembly and function at postsynaptic sites.

At the presynaptic terminal, neurexins interact with CASK, another MAGUK, via PDZ-mediated binding (Fig. 2) [101]. Neurexins can also bind directly to Mints, also via PDZ-mediated interaction [102]. CASK and Mint 1 associate with Veli to form a tripartite complex that acts as a scaffold on which the synaptic vesicle release machinery assembly is based [103, 104]. Furthermore, CASK and/or Mints may couple neurexins with other proteins of the presynaptic machinery known to regulate exocytosis, including presynaptic Ca^{2+} channels, Munc-18, syntaxin, liprin α, and rabphilin 3a (Fig. 2) [37, 102, 105–108]. In addition, neurexins directly bind to synaptotagmins, proteins that regulate neurotransmitter exocytosis [109]. Finally, coupling of neurexins to the actin cytoskeleton has been suggested to be at least partially mediated via interaction of CASK with protein 4.1, a class of proteins that promote the formation of actin/spectrin microfilaments [110]. Thus, CASK may act as a modular adaptor protein that recruits factors important for vesicle exocytosis, organizes them into a macromolecular complex, and couples them to cell adhesion molecules such as neurexins. Other investigations have revealed that α-neurexins associate with neurexophilins, a family of secreted glycoproteins which mediate cell signaling [69, 111]. Another extracellular ligand of both α- and β-neurexins is dystroglycan, a cell surface protein that links the intracellular cytoskeleton to the extracellular matrix and is located at a subset of inhibitory synapses [112–114]. Although the functional relevance of these interactions is uncertain, they have been suggested to regulate adhesion between neurons. Together, these data indicate that neurexins may take part in multiple molecular interactions *in vivo* to regulate a multitude of neuronal processes, ranging from synapse formation/stabilization to neurotransmitter exocytosis and cellular signaling.

Neuroligin, β-neurexin and synapse formation: *in vitro* studies

Synapse formation is a multistep process, triggered after the initial contact of an axon with a dendrite, followed by coordinated differentiation and maturation of the pre- and postsynaptic sites [6, 8]. Differentiation of presynaptic boutons involves recruitment of scaffolding molecules that regulate the formation of a macromolecular complex called the cytomatrix, linking synaptic vesicles and associated fusion machinery with the plasma membrane and cytoskeleton [115]. At the postsynaptic side, several scaffolding proteins have been identified as key regulators of excitatory synapse development through regulation of neurotransmitter receptor clustering, recruitment of adhesion molecules and regulation of dendritic spine formation [2, 4, 87, 88]. Many adhesion systems have been implicated in excitatory synapse development, however, a direct effect on synapse formation by specific adhesion molecules has been only recently discovered.

Insights into a direct role for adhesion molecules in synapse development came from a pioneer study by Serafini and colleagues, which used an assay involving coculture of non-neuronal and neuronal cells to study the minimum molecular requirements for synapse induction [28]. In these experiments, presentation of neuroligin 1 or 2 at the surface of heterologous cells was sufficient to induce functional presynaptic terminals in axons contacting these cells [28]. Neuroligins were sufficient to provoke the accumulation of the synaptic vesicle marker synapsin in pontine axons or granule cells, and these vesicles

could undergo exocytosis in a depolarization-dependent manner [28]. This feature was later demonstrated for SynCAM, another cell adhesion molecule that is able to drive presynaptic differentiation [27]. Electrophysiological recordings of artificial contacts formed between neurons and heterologous cells coexpressing neuroligin 1 with NMDA or AMPA receptor subunits provided further evidence that neuroligins could induce the formation of functional synapses [26, 116].

Neuroligin 1 synaptogenic effects were dependent on its interaction with β-neurexin, since application of soluble β-neurexin blocked presynaptic terminal differentiation [28]. Although required, binding of neuroligin 1 to β-neurexin alone is not sufficient to elicit presynaptic differentiation, since a neuroligin 1 mutant that does not oligomerize but can still bind to β-neurexin fails to induce the formation of new contacts [74]. Thus, both protein oligomerization and association with β-neurexin seem to be required for the synapse-inducing effects of neuroligin.

Consistent with the synaptogenic activity of neuroligins in the coculture assays, neuroligin 1 overexpression in neurons enhanced presynaptic differentiation, triggering clustering of endogenous β-neurexin and increasing the size and number of synapsin, synaptobrevin/VAMP and synaptophysin clusters [74, 87]. Overexpression of neuroligin 1 and 2 on the surface of heterologous cells also induced clustering of both GAD-65 and VGLUT [29]. An increase in synapsin and synaptotagmin clustering was also observed when β-neurexin was directly aggregated using antibodies or purified neuroligin coupled to beads (Fig. 3a) [74]. Other studies reported an increase in spine density upon neuroligin 1 overexpression, suggesting a role for neuroligin in synapse maturation [26, 117]. Overall, these results implicate neuroligins and neurexins in the development of pre- and postsynaptic elements of excitatory synapses.

Other investigations postulated a novel role for neuroligins in inhibitory synapse development. Work done by Prange et al. [87] showed that overexpression of neuroligin 1 in neurons increased the size, number and activity of both excitatory and inhibitory synapses. These effects were mediated through β-neurexin binding, since application of soluble β-neurexin reduced excitatory and inhibitory synapse numbers as well as current frequencies in both neuroligin 1 transfected and untransfected neurons [118]. The involvement of neuroligins in both excitatory and inhibitory synapse development was also reported using protein overexpression and knock-down approaches [117, 118].

The ability of β-neurexins to induce the assembly of postsynaptic proteins normally present at excitatory and inhibitory synapses was elegantly addressed using a coculture strategy, whereby heterologous cells overexpressing β-neurexin were presented to neurons, and the clustering

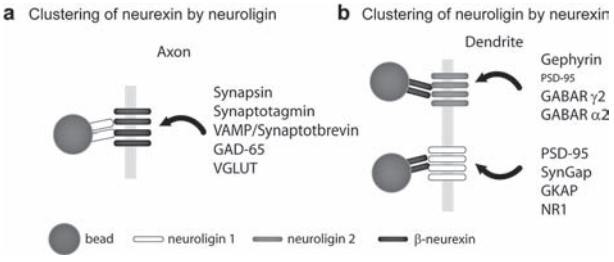

Figure 3. Regulation of protein clustering by neuroligins and β-neurexin. (*a*) Clustering of neurexins by neuroligins is thought to induce recruitment of presynaptic proteins associated with the vesicle release machinery, including synapsin, synaptotagmin, synaptobrevin/VAMP, GAD-65, and VGLUT. (*b*) Neuroligin clustering by β-neurexin triggers accumulation of postsynaptic scaffolding proteins including PSD-95, gephyrin, GKAP and SynGap, as well as excitatory and inhibitory neurotransmitter receptor subunits NR1, $GABA_A$ α2 and γ2. β-neurexin mainly induces aggregation of neuroligin 1 with PSD-95. In contrast, neurexin induces clustering of neuroligin 2 with both PSD-95 and gephyrin, although significantly more with gephyrin.

of excitatory and inhibitory scaffolding proteins and neurotransmitter receptors examined [29]. Presentation of β-neurexin expressed in heterologous cells or bound to beads results in clustering of several postsynaptic proteins normally present at either excitatory or inhibitory contacts (Fig. 3b). These include PSD-95, GKAP, SynGap, NR1, gephyrin, and $GABA_A$ α2 and γ2 receptors. Moreover, β-neurexin presented in these ways enhanced the clustering of ectopically expressed neuroligin 1 and 2. In contrast to these findings, β-neurexin did not induce clustering of endogenous α- or β-dystroglycan, other known binding partners of β-neurexin found at inhibitory contacts [29, 112]. Thus, the initial interaction between neuroligins and neurexins at nascent neuronal contacts was proposed to trigger clustering of specific molecules present at both sides of the synapse. It is important to note that while β-neurexin induced clustering of both PSD-95 and NR1, no clustering of GluR1 or GluR2 was observed [29, 119]. Other studies, however, demonstrated that GluR1 is recruited to neurexin-induced synapses after application of glutamate or overexpression of CAMKII in neurons [119]. Thus, despite the induction of clustering of several molecules, the newly formed contacts by the neuroligin/β-neurexin adhesion complex resemble silent synapses, which become functional at later stages of synapse development upon recruitment of AMPA receptors [120, 121].

Experiments performed by Craig and colleagues revealed that aggregation of specific members of the neuroligin family by neurexins elicit differential effects on the clustering of postsynaptic proteins [29]. Application of purified β-neurexin coupled to beads induced coaggregation of neuroligin 1, 3 and 4 mainly with PSD-95. In contrast, neuroligin 2 coaggregated with both PSD-95 and gephyrin, although significantly more with gephyrin

(Fig. 3b) [29]. This approach provided insights into how β-neurexin interaction with specific members of the neuroligin family may differentially regulate the assembly of postsynaptic proteins at excitatory versus inhibitory contacts. However, overexpression studies of neuroligin 1 in neuronal cells led to some conflicting results. In some studies, an increase in the clustering of PSD-95, Homer and NR1 was reported [74, 117], while other groups observed only a modest increase in PSD-95 cluster density and no significant change in NR1 and PSD-95 cluster size [87, 118]. What could explain these discrepancies? The observed differences may simply be due to the developmental stages examined or variations in the levels of expression and clustering of neuroligins when transfected into hippocampal neurons. In agreement with the second possibility, differences in the extent of clustering of ectopically expressed neuroligins have been reported. Only when expressed at low levels were distinct clusters of these proteins observed. It has therefore been presumed that the reduced clustering of neuroligins when expressed at high levels is due to the insufficient amounts of endogenous PSD-95 to cluster exogenous neuroligins. In support of this notion, an enhancement of neuroligin 1 clustering was observed when PSD-95 was cotransfected with neuroligin 1 [87]. Regardless of the inconsistencies, these results suggest a link between mechanisms involved in trans-synaptic signaling and clustering of scaffold proteins and neurotransmitter receptors at postsynaptic sites.

The studies discussed above were performed using full-length neuroligins that contain both A and B spliced inserts, and therefore are expected to bind only to β-neurexin. As discussed earlier, however, overexpression of a splice variant of neuroligin 1 lacking the insert at splice site B, which also binds to α-neurexin, primarily affected the size of spines and presynaptic terminals, with a less apparent effect on the number of synaptic contacts formed [53]. This new finding suggests that coupling of α-neurexins to specific splice forms of neuroligins influences synapse development. Earlier studies on α-neurexin triple knock-out mice revealed that loss of α-neurexins does not impair prenatal viability; however, these mice die shortly after birth [122]. Moreover, the mutant mice elicited a 50% decrease in the density of symmetric (GABAergic) synapses, whereas no change in the density of asymmetric (glutamategic) synapses was observed. These findings are intriguing and consistent with the new proposed role for neurexins and neuroligins in inhibitory synapse development [29, 41, 87, 117, 118]. However, despite the twofold decrease in the number of VGAT-positive terminals, there was no significant change in the amplitude of spontaneous miniature synaptic responses of GABAergic synapses, indicating that inhibitory postsynaptic currents are not compromised in these mice. Further analysis showed that α-neurexins are required for NMDA but not AMPA receptor activity [123]. Other significant changes in synaptic function in mice lacking α-neurexins include a reduction in voltage-gated Ca^{2+} currents and impairment of Ca^{2+}-regulated neurotransmitter release [122, 124]. Thus, α-neurexins seem to regulate specific aspects of neurotransmitter release and postsynaptic responses. It remains unclear, however, whether the observed synaptic abnormalities are due to disruption of the neurexin-neuroligin trans-synaptic signaling.

Analysis of various mutant forms of neuroligins provided further insight into the potential role for these molecules in synapse maturation/stabilization. Ectopic neuronal expression of neuroligin 1 lacking the extracellular N-terminal region, or having this region swapped with the acetylcholinesterase N-terminal domain to prevent binding to β-neurexin, disrupted alignment of pre- and postsynaptic terminals [117]. Less PSD-95-positive puncta were observed to be colocalized with presynaptic markers when neuroligin lacking the extracellular domain was expressed in hippocampal neurons [87, 117]. Moreover, high levels of neuroligin 2 overexpression disrupted clustering of PSD-95, gephyrin, NR1 and $GABA_A$ receptor. Accordingly, reductions of both miniature excitatory postsynaptic current (mEPSC) and miniature inhibitory postsynaptic current (mIPSC) amplitudes and frequencies were detected [29].

The importance of PDZ-dependent interactions on neuroligin function was also assessed by overexpression of a mutant neuroligin 1 lacking the PDZ-binding motif. Expression of this mutant form resulted in a reduction in the density and size of PSD-95 and GluR2 puncta and a reduction in both amplitude and frequency of AMPA-mediated mEPSC, without affecting inhibitory currents [119]. Other investigations also showed PDZ-dependent interactions are required for recruitment of PSD-95 but not NR1 to the synapse, suggesting that neuroligin-dependent recruitment of PSD-95 and NMDA receptors is mediated by different mechanisms [117]. Surprisingly, neuroligin 1 mutants lacking the N-terminal region or the PDZ-binding site were efficiently recruited into spines, suggesting that associations with β-neurexin or PSD-95 are not essential for targeting neuroligins to postsynaptic structures [125]. Taken together, most of the findings regarding the effects of neuroligins on presynaptic differentiation are consistent with one another, but the effects on the assembly and maturation of postsynaptic elements require further clarification.

Recruitment of neuroligins and neurexins to newly formed contact sites

How are neuroligins and neurexins delivered to contact sites during development? To understand whether there is a sequential order for the recruitment of synaptic pro-

teins and whether they are recruited as preassembled complexes or as individual molecules has been an area of intense research. Recent studies have shown that synapse formation involves the rapid delivery of preassembled transport packets containing presynaptic proteins to nascent contact sites. Studies from several laboratories have suggested that vesicular delivery of proteins plays a prominent role in this process at the presynaptic side [3, 5, 6, 8, 126, 127]. The fusion of vesicles carrying structural components of the presynaptic active zone, such as piccolo and bassoon, has been observed shortly after initial contact [115]. Recruitment of another population of precursor presynaptic vesicles containing proteins important for active neurotransmitter release, such as synaptobrevin/VAMP and synaptophysin, has also been observed shortly after initial contact formation [128]. Considering their potential role in synapse initiation, adhesion molecules are thought to be the driving force for the recruitment of these preformed complexes. However, little is known regarding the processes that control the delivery of adhesion molecules to nascent contact sites. Live imaging of NCAM, an adhesion molecule thought to participate in the early events in synaptogenesis, revealed that trans-Golgi network-derived vesicles containing NCAM move along neurites and accumulate within several minutes after contact formation [129]. Thus, regulated delivery of specific adhesion molecules via vesicular transport-dependent processes may be involved in controlling synapse specificity and stability. Alternatively, adhesion molecules may initially traffic to the plasma membrane, and once an encounter between an axon and a dendrite occurs, they are retained and clustered by factors that regulate their lateral movement at the newly formed contact site.

Analysis performed in young hippocampal neurons (5–8 days *in vitro*) revealed that neuroligin 1 partially exists in a complex containing several scaffolding proteins including PSD-95, GKAP and Shank at non-synaptic sites [86]. Time-lapse imaging revealed that stationary clusters of the preformed complex of scaffold proteins containing neuroligin 1 preceded the recruitment of presynaptic vesicle proteins and the machinery required for active recycling of neurotransmitter [130]. In this case, the presence of postsynaptic proteins such as neuroligin 1 may have accelerated the recruitment of presynaptic proteins important for vesicular release [86]. In contrast with these results, however, previous studies demonstrated that the recruitment of PSD-95 occurs after the establishment of an active presynaptic terminal [9, 10, 128]. Although the role of adhesion complexes was not studied, the presumed role of neuroligin 1 would be to recruit PSD-95 to these sites. Further studies are required to monitor in more detail the time course of recruitment of these molecules at nascent contacts to determine when neuroligins and neurexins come into play. The development of new techniques to visualize the movement of these molecules in live cells during synapse development will help address these issues [131, 132].

Modulation of excitatory and inhibitory synapse development

Immunohistochemical analysis has revealed differential localization of neuroligins in the brain. Endogenous neuroligin 1 is mainly present at excitatory synapses, colocalizing with AMPA receptor subunits GluR2/3 [75]. In contrast, neuroligin 2 is mainly detected at inhibitory synapses in rat brain, colocalizing with $GABA_A$ receptors [76]. Differential subcellular localization of neuroligin 1 and 2 has been described in cultured cells as well [29, 117, 118]. Differential association of neuroligins with a unique subset of scaffolding molecules has been hypothesized to modulate the balance between excitatory and inhibitory synapse development. In support of this idea, coexpression of PSD-95 with neuroligins modulates the clustering of neuroligins as well as the type of synapses they induce [87, 118].

Coexpression with PSD-95 blocked the increase in mIPSC frequencies elicited by neuroligin 1, resulting in an overall increase in the excitatory/inhibitory (E/I) synaptic ratio [87]. These changes can be explained by the observed shift in the localization of endogenous neuroligin 2 from inhibitory to excitatory synapses in neurons overexpressing PSD-95 [29, 118]. In contrast, small interference RNA (siRNA)-mediated knock down of PSD-95 resulted in a shift in the localization of neuroligin 1 at inhibitory contacts [86, 87]. Taken together, these results suggest that PSD-95 may sequester members of the neuroligin family to excitatory synaptic contacts at the expense of inhibitory synapses. This is consistent with the fact that all neuroligins contain a PDZ-recognition motif that binds to PSD-95 [82]. However, considering the capacity of both neuroligin 1 and 2 to bind to PSD-95, how endogenous neuroligin 1 and 2 are differentially targeted to excitatory and inhibitory synapses, respectively, remains obscure. One possibility is that neuroligin 1 has a higher binding affinity than neuroligin 2 for PSD-95. Another possibility is that yet unknown adaptor proteins compete with PSD-95 for binding to neuroligin 2, sequestering it at inhibitory contacts. Interaction with other proteins, such as gephyrin, located at inhibitory synapses may also influence the distribution of neuroligin 2 at inhibitory contacts. Further studies are required to identify molecules that interact with the intracellular domains of neuroligin family members.

When neuroligin 1, 2 and 3 were knocked down by siRNA either individually or collectively, a reduction in both excitatory and inhibitory presynaptic terminals was observed [117]. Altered synapse maturation was also observed, as

assessed by decreased spine number and reduced GluR1 puncta density [117]. However, simultaneous knock down of all three isoforms of neuroligins resulted principally in a disruption of inhibitory transmission, with only modest effects on excitatory synaptic transmission [117]. These results suggest that neuroligins may play a more crucial role in the formation/stabilization of inhibitory synapses. Another possibility is the existence of redundant adhesion systems at excitatory synapses that compensated for the loss of neuroligins. Thus, mounting evidence indicates that expression levels and localization of neuroligins can alter the excitatory/inhibitory balance *in vitro.* Whether changes in expression levels of neuroligins and/or PSD-95 alter E/I *in vivo* remains to be determined.

Diseases associated with neuroligin family member dysfunction

The emerging paradigm implicating neuroligin family members and PSD-95 in dictating the ratio of E/I synaptic contacts is a very exciting prospect, providing a mechanism for balancing excitation and inhibition in the brain [13, 39, 41, 42]. This process is critical for proper brain function, since alteration in the E/I ratio during brain development can lead to abnormal synaptic connectivity and function, resulting in severe neurological impairments. Recently, disruption of synaptic connectivity and/or activity has been implicated in abnormalities associated with neurodevelopmental disorders such as fragile X mental retardation, Asperger syndrome and autism disorder. Autism disorder and Asperger syndrome are classified as pervasive developmental disorders, and are characterized by varying degrees of communication skills and social interactions, as well as repetitive and stereotyped patterns of behavior. The timing of abnormal early postnatal brain development in autism suggests defects in neuronal connectivity. The association with synaptic abnormalities is further strengthened by the fact that fragile X syndrome, a disorder of known genetic cause, shows substantial symptomatic overlap with autism. Moreover, the high concordance of autism in monozygotic as compared with dizygotic twins strongly points to an important role of genetics in autism.

The behavioral and cognitive deficits found in autistic patients may result from alterations in the expression of synaptic proteins that control neuronal circuitry implicated in sensory, mnemonic, social and emotional information processing. Neuroimaging and neuropathological studies of these circuits in autistic patients have provided important insights into the etiology of autism, leading to a new model that could explain the observed dysfunctions. This model postulates that a change in the ratio of E/I in neural circuits involved in information processing may underlie the dysfunctions characteristic of autism [133]. This imbalance in favor of more excitable (more weakly inhibited) cortex is associated with less differentiated cortex, which is prone to epilepsy, perceptual anomalies, as well as atypical memory, cognitive style, motor stereotypy and repetitive behavior. This model is substantiated by several examples from animal models showing how the imbalance of the E/I inputs could lead to cognitive or perceptual anomalies reminiscent of autism manifestations. For example, recent investigations into the development of auditory systems in rat have shown that sound representation in the brain is determined during a critical 1-week period when the environmental auditory exposure determines the 'tonotopic map' [134, 135]. Under some experimental conditions, such as continuous or modulated noise, the critical period will be either interrupted or indefinitely protracted, leading in both cases to 'noisy' cortical development, with proneness to epilepsy and possibly abnormal auditory experience. Other studies have shown that during development, the balance between excitation and inhibition governs the establishment of sensory system projections, including the onset of the critical period [136]. Considering the potential role of neuroligins in dictating the E/I ratio, aberrations in the expression of these proteins may be associated with these disorders.

Consistent with this, rearrangement of chromosomal regions harboring neuroligin genes have been linked to autism [137–143]. Other investigations have revealed that mutations in neuroligin 3 and neuroligin 4 are associated with autism [78, 143–145]. A summary of these studies is presented in Table 1. In one study, a missense mutation consisting of a substitution of Arg 451 for Cys (R451C) was identified in neuroligin 3 [78]. In the case of neuroligin 4, a novel insertion at position 396 (D396X) was found to result in a frame shift and premature translational termination [78]. Support for the involvement of neuroligin 4 in autism was provided by another genetic study of a large French family with members affected by non-specific X-linked mental retardation, with or without autism [144]. In this study, another frame shift mutation in neuroligin 4 was identified. In this case, a 2-base-pair deletion was found to generate a stop codon in the middle of the protein. The fact that this mutation was found in both autistic and mentally retarded patients indicates that autism and mental retardation are associated with defects in synaptic balance. Finally, another recent study found missense mutations in the neuroligin 4 gene in four unrelated autistic patients [145]. These mutations, which are located within the coding sequence of the protein, may also contribute to autism susceptibility.

In vitro studies have provided further understanding of how mutations in neuroligins associated with autism might alter cellular mechanisms and lead to synapse dysfunction. When expressed in heterologous cells, a defect in cell surface transport of some of these mutants was

Table 1. Chromosomal aberrations associated with autism in regions that harbor *neuroligin* genes.

Name of protein symbol of the gene	Chromosomal location	Linkage to autism	References
Neuroligin 1 *NLG*	3q26-3	Chromosomal abnormality detected on Chr. 3q in children with autism disorder.	138
		Linkage on Chr. 3q was replicated at least once in members of families with autism disorder or Asperger syndrome.	139, 140
Neuroligin 2 NLG2	17p13.2*	Chromosomal abnormalities detected on Chr. 17p in patients with autism disorder and mental retardation.	141
		Report linkage on Chr. 17p in sibships affected with autism disorder. *17p13 harbors a cluster of genes that represent good candidates for autism: *NLG2, DLG4* (encoding PSD-95) and the gene coding for GABA receptor-associated protein (GABARAP), a protein implicated in GABAR clustering.	142
Neuroligin 3 *NLG3*	Xq13.1	Two brothers with autism disorder or Asperger syndrome were found with a point mutation (R451C).	78
Neuroligin 4 *NLG4*	Xp22.33	De novo chromosomal deletions detected in three males with autism.	143
		Two brothers with autism disorder or Asperger syndrome were found with a frameshift mutation (D396X) generating a stop codon.	78
		A 2-base-pair deletion found (1253delAG) generating a stop codor.	144
		Four missense mutations found in unrelated patients with autism disorder (G99S, F378R,V403M, R704C).	145

detected [146–148]. This effect was correlated with an increase in their accumulation in the endoplasmic reticulum, presumably due to protein misfolding [147, 148]. When expressed in hippocampal neurons, both mutants showed signs of defective trafficking and loss of ability to promote presynaptic differentiation [147]. Further biochemical analysis of the neuroligin 3 D396X mutant reveals that this mutation significantly reduces the affinity of neuroligin 3 for neurexin 1β [146]. A related study on neuroligin 3 carrying the autism-associated mutation confirmed the previous reports of defective trafficking to the cell surface [149]. Notably, however, a small percentage of these mutants were still trafficked to the surface and their synapse-inducing ability was preserved, indicating that autism-associated mutation does not completely eliminate synaptogenic activity of neuroligins. Other investigators have shown that introduction of a point mutation in neuroligin 1, similar to the neuroligin 3 mutation (R451C) associated with autism, can induce an increase in NMDA current amplitude and that this change is associated with enhanced NMDA receptor clustering [150]. Taken together, these results suggest that neuroligin mutations are likely to be important for explaining the synaptic defects associated with autism spectrum disorders and mental retardation.

Despite these studies, the implication of neuroligins in autism has been debated. Other genetic screens of autistic patients have failed to identify any mutations in the coding regions of neuroligin genes, suggesting that neuroligin mutations may be responsible for a only small fraction of autism cases [142, 151–154]. It is tempting to speculate that mutations in proteins leading to altered synaptic balance rather than neuroligins per se are involved in the manifestation of this disorder.

Other potential roles of neuroligins at the synapse

Most of the synaptogenic effects discussed earlier point to a critical role for neuroligins in synapse specificity, induction, stabilization and maturation (Fig. 4). Does the neuroligin/neurexin adhesion complex have a role in target recognition and specificity *in vivo* (Fig. 4a)? Neurexins are highly polymorphic, and the differential association of specific splice forms with neuroligins could be critical for regulating synapse specificity. However, the number of neuroligin splice variants is more limited and, therefore, the principle function of the neuroligin/neurexin complex seems unlikely to be target recognition. Another open question is whether neuroligin effects are mainly restricted to early events in synapse development or participate at later stages in events that control synaptic transmission and plasticity? One example of multifunctional cell adhesion molecules at the synapse is the cadherin family. Cadherins, with their multiple isoforms and splice variants, and expression that varies with neuronal type and developmental stage, have been implicated in mediating target specificity, synapse maturation and spine morphogenesis [25, 155–161]. Interestingly, N-cadherin expression is regulated by synaptic activity, suggesting that this molecule plays a role in activity-dependent modifications at the synapse [162]. Other studies on specific adhesion systems such as NCAM, integrins, and ephrins have also provided insights into the involvement of these molecules in trans-synaptic signaling cascades associated with synaptic plasticity [163–167]. Future investigations are needed to assess whether expression and localization of neuroligin/neurexin is modulated by synaptic activity and whether this adhesion complex regulates synaptic plasticity (Fig. 5).

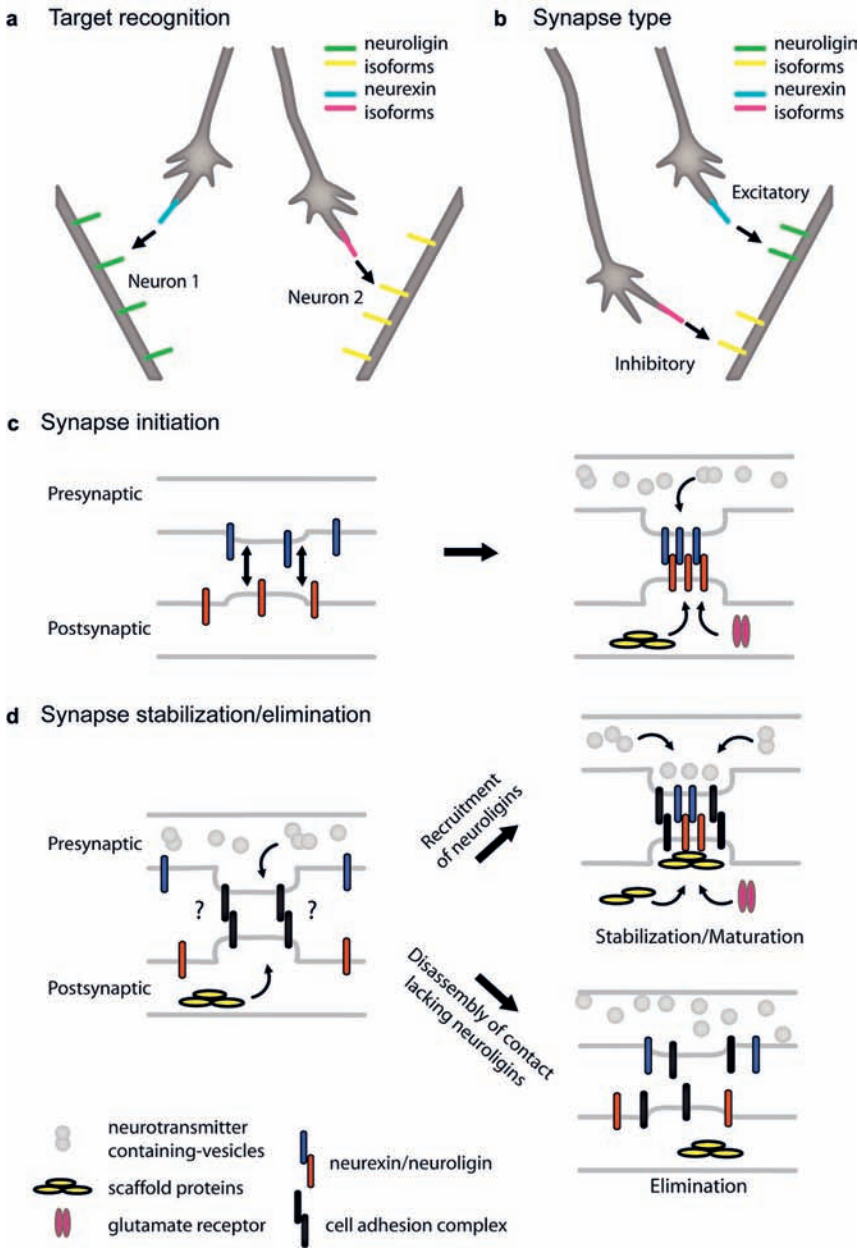

Figure 4. Proposed roles for neuroligins and neurexins in synapse development. (*a*, *b*), Putative role of neuroligin/neurexin interaction in regulating target recognition (*a*) and synapse specificity (*b*). A combination of specific forms of neuroligins and neurexins may allow the recognition of a particular type of neuron or even a specific location on the target neuron or determine the type of synaptic contacts formed, for example, excitatory versus inhibitory. (*c*) Initial contact between a dendrite expressing neuroligin and an axon expressing neurexin (left panel) triggers clustering of neuroligin and neurexins, as well as the coordinated recruitment of presynaptic protein packets and postsynaptic scaffold proteins (right panel). (*d*) Another potential scenario is that other cell adhesion molecules would initiate contact formation, followed by recruitment of neuroligins to the synapse. In this case, neuroligins may only be involved in synapse stabilization and maturation. The absence of neuroligins may lead to contact destabilization and ultimately synapse elimination.

Concluding remarks

Several new findings obtained from studies utilizing either *in vitro* assays or performed in neuronal cultures have provided new insights into the potential roles for neuroligins and their bindings partners in the development of excitatory and inhibitory neuronal contacts. These studies have generated much excitement regarding the possible involvement of these molecules in controlling the balance between excitatory and inhibitory synapses. Future *in vivo* experiments are clearly required to tease out the role of neuroligins in different aspects of synapse development, including contact initiation, target recognition, synapse stabilization/maturation and plasticity. However, one must bear in mind that synapse formation and maturation likely rely on multiple parallel or chronologically sequential ad-

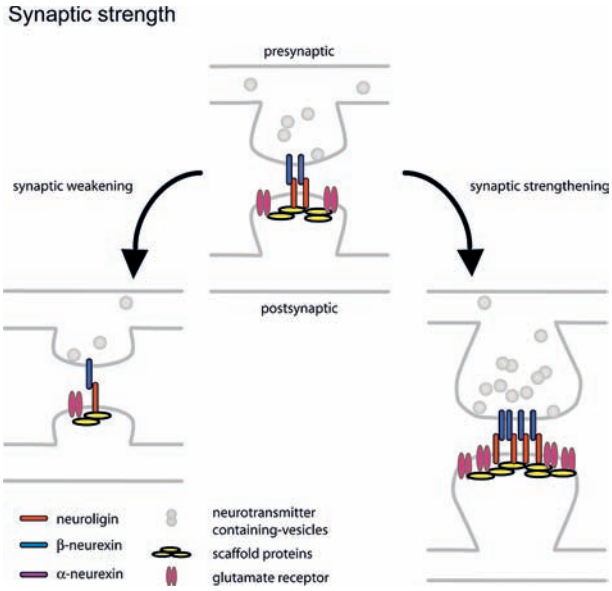

Figure 5. Putative role of neuroligins and neurexins in the modulation of synaptic strength. An increase in synaptic activity may result in an increase in the recruitment and/or stabilization of the neuroligin/neurexin complex at the synapse. Trans-synaptic signaling through these molecules may lead to further recruitment of critical elements to pre- and postsynaptic sites that modulate synapse activity and ultimately lead to enhanced synaptic strength (right). Alternatively, a decrease in activity may result in loss or uncoupling of the neuroligin/neurexin complex from the synapse, resulting in reduced synaptic activity (left).

hesion pathways, which will surely introduce additional levels of complexity into currently existing models. Elucidation of these cooperative cell adhesion systems will undoubtedly lead to a more complete understanding of synapse development and function. Notably, animals mutant for neuroligin 1, neuroligin 2 or both, have been generated and these are viable, fertile and show no apparent behavioral abnormalities [75, 76]. These results may be attributable to functional redundancy of neuroligin isoforms or to the existence of compensatory mechanisms, mediated through other trans-synaptic adhesion complexes. At the same time, these results raise important questions about the importance of neuroligins in synapse formation. Further analysis of animals mutant for all known neuroligins may help clarify the role of these proteins in synapse development.

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