Chapter 5

# Ligand-Induced Cell Adhesion in Synapse Formation

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## 1. Introduction

Construction of the neural netwoks depends largely on precise contacts between neurons and non-neuronal cells. Numerous studies have described different types of adhesive interactions between cells in the nervous system. These include adhesive contacts between neural cell bodies, axonal attachments to glial cells, axon fasciculation, connection between pre- and postsynaptic specializations as well as to cells outside the nervous system. Although some of the molecules that mediate each of these types of neural adhesive contacts have been characterized, some remain unknown.

Neuronal synapses can be considered as a specialized type of cell-cell interaction that mediates communication between neurons and their target cells. It involves the interaction between two asymmetric partners, the presynaptic specialization, where the synaptic vesicles release neurotransmitters and the postsynaptic density, which contains receptors and adapter scaffold proteins that transduce the neurotransmitter signal [1]. As at other cell-cell junctions, such as epithelial tight junctions or the immune synapse, synaptically-localized neural cell adhesion molecules are not merely static structural components but are often dynamic regulators of synapse function.

In the last years numerous studies provide new insights into the role of adhesion molecules in the formation, maturation, maintenance, function and plasticity of synaptic contacts. Several cell adhesion molecules have been involved in synapse development, including, cadherins, proteocadherins, integrins, NCAM, L1, Fasciclina, Syg, Sidekicks, SynCam, Neurexin-Neuroligin, LRRTM, GDNF/GFRα, Neurexin/Cbl1/GluRδ2, between others [2]. *Trans*-synaptic cell adhesion molecules are particularly attractive candidate mediators of synapse formation because of their potential to bidirectionally coordinate functional and morphological synapse differentiation [3,4].

During neuronal development, specific synaptic circuits are generated by synapse formation between the appropriate pre- and postsynaptic partners and aberrant connectivity can lead to nervous system disorders. The accuracy of synapse formation is fundamental for the normal brain development and depends in part on the controlled spatial and temporal expression of selective adhesion molecules on neuronal surface.

## 2. Adhesion at the synapses

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, they are important elements in the *trans*-cellular communication mediated by synapses. During the last years, some adhesion complexes, which are involved in the formation, maintenance and modulation of synaptic contacts, have emerged. Three particularly interesting molecular systems of pre- and postsynaptic partners that interact *in trans* across the synaptic cleft, have been described: Neurexin-Neuroligin, LRRTM and SynCam.

### 2.1. Neurexins and neuroligins

Neurexins (Nrxns) and Neuroligins (Nlgns) are the best characterized synaptic cell adhesion system. Neurexins were originally discovered as receptors for α-latrotoxin, a vertebrate-specific toxin present in the black widow spider venom that binds to presynaptic receptors and induces massive neurotransmitter release [5]. There are two types of Nrxns, a longer α-Nrxn (α-Nrxn) and a shorter β-Nrnx (β-Nrxn) isoforms. While α-Nrxn have six extracellular LNS domains (Laminin/ Neurexin/ Sex hormone-binding globulin-domain) with three intercalated EGF-like domains, β-Nrxn only contains a single LNS domain [6,7,8]. Immunofluorescence and subcellular fractionation analysis indicate that Nrxns are located on presynaptic terminals [6,9].

Mammals contain three Nrxn genes (Nrxn1-3), each of which directs the transcription of α- and β-Nrxns from independent promoters [10]. Neurexins are evolutionary conserved and pan-neuronally expressed [10,11]. Homologues of neurexin genes have been described in low vertebrates such as *Danio rerio* [12], and invertebrates such as *Drosophila melanogaster*, *Caenorhabditis elegans*, honeybees and *Aplysia* [10,13,14,15]. In mammals, alternative splicing of Nrxns can generate thousands of alternatively spliced mRNA transcripts. The ectodomain of α-Nrxns contains five sequences that can be alternatively spliced (S1-5), two of which are also present in β-Nrxns (S4 and S5) [10,11]. Some of these splice sites are localized in the Nrxn binding domain. Interestingly, Nrxn alternative splicing is regionally regulated during development and by neural activity [11,16], and plays an important role in modulating its function at synapses. Indeed, Nrxn distribution to excitatory and inhibitory synapses seems to be regulated by alternative splicing. It has been reported that β-Nrxn without the S4 sequence (-S4) induce differentiation of excitatory synapses while β-Nrxn containing the S4 insert (+S4) promote differentiation of inhibitory synapses [17,18]. Furthermore, α-Nrxn also strongly promotes differentiation of inhibitory synapses [17].

Neuroligins have been identified as endogenous Nrxns ligands [19,20]. As Nrxns, Neuroligins (Nlgns) are type I membrane proteins that consist of an extracellular region, involved in *trans*-synaptic interactions, a single transmembrane sequence and a small cytoplasmic domain that contain a PDZ-domain binding sequence that recruits PSD-95 and other PDZ-domain proteins [21]. The extracellular region contains a domain homologous to acetylcholinesterase, without its enzymatic activity. All Nlgns are enriched in postsynaptic densities. The human genome expresses five Nlgns isoforms (Nlg1-5), and rodent genome contains only 4 isoforms (Nlg1-4). Homologues of Nlgns have been identified in invertebrates including *Drosophila melanogaster* [22,23], honeybees [13,24], *Caenorhabditis elegans* [25] and *Aplysia* [14,15]. In mammals, Nlgns contains two alternative splice sites referred to as SA (in Nlgn1-3) and SB (in Nlgn-1) [20,26]. In contrast to Nrxns, Nlgns are specifically localized to particular synapses. Several studies revealed that Nlgn1 and Nlgn2 are exclusively localized to excitatory and inhibitory synapses respectively. Nlgn3 has been described to be present at both inhibitory and excitatory synapses in hippocampal cells [27,28,29,30,31], while Nlgn4 appears to be localized to inhibitory synapses in some tissues such as, retina, spinal cord, and several lower brain regions; and to excitatory synapses in some tissues such as, hippocampus and cortex [27].

Nrxn-Nlgn complex has been involved in the formation, maturation and function of vertebrate synapses. The evidence indicates that Nrxn-Nlgn bind each other by their extracellular domain to promote adhesion between pre- and postsynaptic specializations, recruiting pre- and postsynaptic molecules to form a functional synapse. Cell based assays of synapse assembly showed that contact of dissociated neurons with Nlgn-expressing fibroblasts can induce the formation of functional presynaptic specializations by recruiting components of the presynaptic machinery in co-cultured neurons [32], while contact of neurons with Nrxn expressing non-neuronal cells can induce postsynaptic differentiation and clustering of postsynaptic receptors in contacting dendrites [31,33].

Recent studies indicate that alternative splicing of *Nrxn* and *Nlgn* mRNA may play an important role modulating the assembly and synapse properties of Nrxn-Nlgn complex [26]. As it has been previously mentioned, Nrxns and Nlgns contain sequences that can be alternatively spliced. Some of these splice sites are localized in the Nrxn-Nlgn binding interface, placing them in a relevant position to modulate Nrxn-Nlgn engagement. It has been proposed that alternative splicing of Nrxns underlies an adhesive code and/or synapse-specific functions [18,34,35]. The evidence indicates that the sequence S4 of Nrxn and insert B of Nlgn have a crucial role in the Nrxn-Nlgn interactions. It has been described that the presence of the insert S4 in β-Nrxn (+S4) strongly reduces binding to Nlgn1 containing the insert B (+B) [26], indicating that Nlgn1(+B), the most common form of Nlgn1, interacts preferentially with β-Nrxn (-S4). Moreover, the presence of insert B inhibits the binding of Nlgn1 to α-Nrxns [36]. On the other hand, the alternative splicing of the segment S4 in Nrxn controls interactions with other Nrxn ligands, such as leucine-rich repeat proteins (LRRTMs) and the Cbln1-GluδR2 complex (See bellow).

The *trans*-synaptic Nrxn-Nlgn complex promotes the assembly and maturation of pre-and postsynaptic machinery. Although the presynaptic signaling events induced by Nrxns are

currently not clear, the evidence indicates that neurexins bind directly to the presynaptic scaffolding proteins CASK, MINT1 and to protein 4.1; and could therefore recruit elements of the presynaptic release machinary [37,38]. In agreement with this, it has been shown that RNA interference (RNAi)-mediated supression of the Nlgn-1, -2, -3 in neuronal cultures reduces the number of excitatory and inhibitory synapses [18]. Conversely, Nlgn1 overexpression in neurons has been found to increase the formation of mature presynaptic boutons, enhace the size of the pool of recycling synaptic vesicles and the rate of synaptic vesicle endocytosis [39,40]. Regarding to the postsynaptic consequences of Nrxn-Nlgn interaction, it was described that in excitatory synapses, this adhesion complex is able to capture AMPA receptors through PSD-95 scaffolding proteins [41]. NMDARs are also recruited to Nrxn-Nlgn complex, but this recruitment does not depend on the presence of PSD-95 [42]. On the other hand, studies on Nrxn-Nlgn interaction at inhibitory synapses indicates that the contact between α-Nrxn and Nlgn2 induces clustering of Nlgn2 and recruitment of the post-synaptic scaffolding protein Gephyrin to inhibitory synapses [31]. Complex formation between Nlgn2, Geophyrin and a brain specific GDP/GTP exchange factor, Collybistin, recruits GABA and Glycine receptors to nascent inhibitory synapses. Interestingly, deletion of Nlgn2 in mice perturbs GABAergic and glicinergic synaptic transmission and leads to a loss of postsynaptic specializations specifically at inhibitory synapses [43].

Although the *in vitro* evidence indicates that Nrxn-Nlgn complex induces synapse formation, the analysis of different Nlgn and Nrxn knockout mice are controversial. *In vivo* deletion of all Nrxn or of Nlgn1-3 do not substantially affect synapse formation [30,44], but impair synapse function, suggesting that α-Nrxn and Nlgns are cell adhesion molecules that play an essential role in synapse maturation but are not essential for synapse formation.

## 2.2. LRRTM

The LRRTM gene family was first described in 2003 [45]. The LRRTM family has four members (LRRTM 1-4) that share similar domain structure with an extracellular domain containing ten extracellular leucine-rich repeats that mediate protein-protein interactions, followed by a single transmembrane domain and a short c-terminal sequence containing a class I PDZ-domain-binding motif. Human and mouse LRRTMs are highly conserved and orthologous genes exist in other vertebrates, but not in invertebrates [45].

*In situ* hybridization, RT-PCR and immunofluorescence analysis showed that LRRTMs are predominantely expressed in the nervous system and that each LRRTM present a specific and partially overlapping expression pattern [45,46,47]. The four LRRTM are expressed in neurons of the hippocampus, cerebral cortex and in the striatum. LRRTM1 and LRRTM2 are also highly expressed in the thalamus. In contrast, neither LRRTM3 nor LRRTM4 are expressed in these structures. The structural similarities and expression patterns of LRRTMs indicates a possible functional redundancy between them [45].

All four LRRTMs family members are post-synaptic localized and when expressed in non-neuronal cells co-cultured with hippocampal neurons they can induce presynaptic differentiation in contacting axons. LRRTM1 and LRRTM2 selectively promote excitatory,

but not inhibitory presynaptic differentiation [46]. In addition, Wit et al (2009) demonstrated that LRRTM2 can interact with the post-synaptic protein PSD-95 and regulate surface expression of AMPA receptors [48].

Independent studies have shown that post-synaptic LRRTM1 and LRRTM2 bind specifically to presynaptic α and β-Nrxn lacking an insert at S4 [49]. Thus, whereas Nlgns bind Nrxn containing or lacking an insert in splice site S4, LRRTMs bind only Nrxns lacking an insert in this splicing site [48,50]. This ability to regulate interaction Nrxn-Nlgn and Nrxn-LRRTM provides an intringuing mechanism for regulating synaptic specificity.

Consistent with the effects of LRRTM on neuronal connectivity, deletion of LRRTM1 in mice revealed altered distribution of the vesicular glutamate transporter vGlut1 *in vivo* [46]. Furthermore, it was demonstrated that lentivirus-mediated knockdown of LRRTM2 *in vivo* decreases the strength of glutamatergic synaptic transmission. Conversely, LRRTM2 overexpression resulted in an increase of excitatory synapses [48,51].

### 2.3. SynCAM

The SynCAM (Synaptic Cell Adhesion Molecule) family comprises four genes encoding proteins (SynCAM1-4) with three inmuonoglobulin (Ig)-like domains, a single transmembrane region, and a short cytosolic tail with a PDZ type II motif. SynCAM proteins are predominantly expressed in the brain and localize to pre- and postsynaptic sites [52,53].

All SynCAMs are expressed mostly by neurons during the peak period of synaptogenesis around the second postnatal week and remains expressed throughout adulthood in the hippocampus [52].

SynCAM proteins are present at presynaptic and postsynaptic specializations and are involved in homophilic and heterophilic interactions via the extracellular (Ig)-like domains. Interestingly, SynCAM1, 2 and 3, but not SynCAM4, can associate *in trans* by homophilic interactions. However, the evidence indicates stronger heterophilic interactions of SynCAM1/2 and SynCAM3/4 more than homophilic adhesion between each other [54]. It has been shown that heterophilic adhesion complex of SynCAM1/2 drives presynaptic terminal formation in cultured neurons, increasing the number of excitatory synapses [54]. In agreement with a functional role of SynCAM1 in neuronal connectivity, it has been demonstrated that elevated expression of SynCAM in a transgenic model increases functional excitatory synapse number [55]. Conversely, SynCAM1 knockout mice exhibited fewer excitatory synapses. Interestingly, SynCAM1 can alter the plasticity of synapses once they are formed. Thus, SynCAM1 overexpression has been shown to abrogate long term depression (LTD), while it loss increased LTD [55].

## 3. Control of synapse formation by ligand-induced cell adhesion molecules (LICAM)

During the last years, a novel mechanism of ligand-induced cell adhesion has been described. Unlike other cell adhesion systems, which involve the simple encounter of

membrane associated cell adhesion molecules *in trans*, ligand-induced cell adhesion is mediated by membrane-associated proteins but is dependent on the presence of its soluble ligand. This feature may allow a more dynamic response to external stimulus involved in synapse development.

### 3.1. GDNF and GFRα1

The Glial cell-line Derived Neurotrophic Factor (GDNF) and its glycosylphosphatidylinositol (GPI)-anchor receptor, GFRα1, represent the first example of this new mechanism of cell-adhesion [56]. In this system, GDNF, is able to mediate *trans*-homphilic cell adhesion between cells expressing its receptor, GFRα1. The receptor involved in this process, the GFRα1, can be considered as a ligand-induced cell adhesion molecule (LICAM). The molecular bases underlying *trans*-homphilic interaction mediated by GDNF and GFRα1 is not clear yet. The domains of GFRα1 underlying its LICAM activity have been analyzed using deletion mutants of the receptor. This study revealed that the GFRα1-mediated cell adhesion requires the presence of an intact ligand-binding domain in both interacting partners. In principle, GDNF, as a dimeric protein could promote *trans*-homophilic interactions between receptor-expressing cells. On the other hand, GDNF could act through an allosteric mechanism.

During the last years, numerous studies have shown that GDNF-family ligands contribute to synapse development and maturation [57,58]. The developmental expression pattern of GFRα1 and its ligand, GDNF, during the period of hippocampal synaptogenesis as well as its subcellular localization at pre- and postsynaptic specializations indicated a possible role of GDNF-GFRα1 complex in the formation of neuronal synapses by inducing *trans*-synaptic homophilic cell adhesion. Indeed, microspheres containing GFRα1, mimicking its postsynaptic localization, were able to induce presynaptic differentiation on hippocampal and cortical neurons cultured in the presence of GDNF. This effect was evidenced by recruitment of vesicle-associated synaptic protein, neurotransmitter transporters and activity-dependent vesicle recycling on the hippocampal axons at the sites of contact [56]. Intringuinly, the presynaptic maturation triggered by GDNF and GFRα1 was independent of the canonical receptor Ret and only partially dependent on the neural cell adhesion molecule, NCAM, indicating the existence of an additional signaling molecule involved in this process (Figure 1A)[56,57,59,60]. Whether postsynaptically localized GFRα1 may contribute to postsynaptic maturation remains to be explored. Thus, the ability of GDNF to trigger *trans*-homophilic interactions between GFRα1 molecules represents the first example of regulated cell-cell interactions and a new synaptogenic mechanisms that combines soluble and membrane bound molecules by inducing conformational changes that reorient and expose determinants involved in *trans*-homphilic binding [56,57].

### 3.2. Cerebelin-GluRδ-neurexin

More recently another example of ligand-induced *trans*-synaptic adhesion interaction has been described. Uemura *et al* (2010) described that the postsynaptic glutamate receptor

(GluR)δ2 interacts with the presynaptic β-Nrnx through the presynaptically secreted glycoprotein, Cerebelin 1 precursor protein (Cbln1) [61].

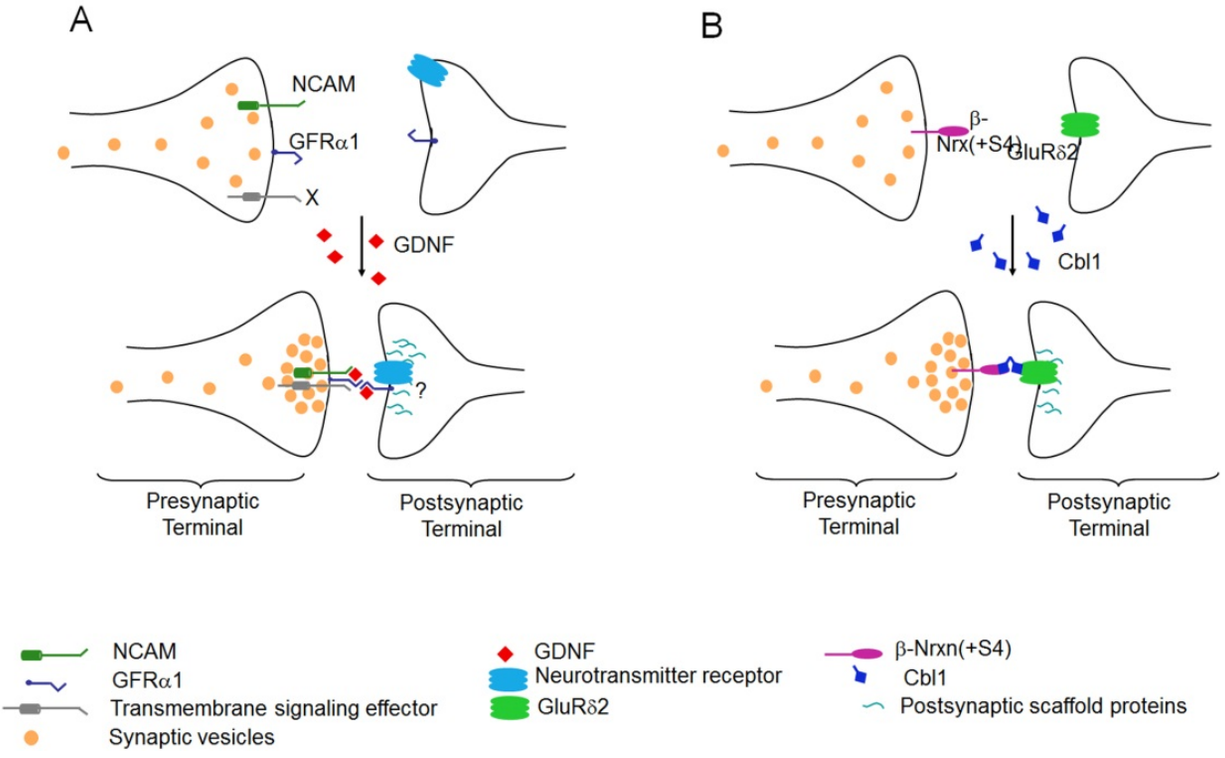
Based on its amino acid sequence, GluRδ2, is a member of the ionotrophic glutamate receptor family, which plays an essential role in cerebellar Purkinje cells (PC) synapse formation [61,62]. The synaptogenic activity induced by GluRδ2 can be reproduced *in vitro* using primary cultures of cerebellar granule cells (GC) and the extracellular N-terminal domain of GluRδ2 [61] indicating that this domain is critical for its synaptogenic activity. Binding studies demonstrated that postsynaptic GluRδ2 interacts with presynaptic Nrxns, which are known to play a crucial role in presynaptic organization. But this interaction is established through the presynaptically secreted glycoprotein Cbln1. Interestingly, the synaptogenic activity of GluRδ2 is abolished in cerebellar primary cultures from Cbln1 knockout mice and is restored by recombinant Cbln1. In agreement with this, Cbln1-null mice show similar behavioral and physiological phenotypes to those of GluRδ2-null mice confirming that Cbln1 and GluRδ2 are involved in a similar signaling pathway [63]. Direct binding experiments demonstrated that Cbln1 acts as a divalent ligand for postsynaptic GluRδ2 and presynaptic Nrxns, representing a new example of ligand induced *trans*-heterophylic synaptic adhesion. The resulting complex, Nrxn/Cbl1/GluRδ2, mediates synapse formation between cerebellar granule cells and Purkinje cells (Figure 1B). In accordance with this, the amino terminal domain of GluRδ2 and the extracellular domain of β-Nrxn1 suppressed the synaptogenic activity of Cbln1 in cerebellar primary cultures *in vivo* indicating that the interaction of GluRδ2, Cbln1 and Nrxn is essential for cerebellar synapse formation [61].

The evidence indicates that Cbln1 interacts with different subtypes of β-Nrxn and α-Nrxn containing the S4 insert, but not to subtypes lacking the S4 insert, to induce synaptogenesis in cultured cerebellar, hippocampal and cortical neurons. Interestingly, α-Nrxn containing the S4 insert binds to Cbln1 [62] but does not bind to any Nlgs or LRRTMs. Another distinctive feature of the Nrxn/Cbln1/GluRδ2 complex is that it is insensitive to the extracellular $Ca2^{+}$ concentration [62]; while binding of Nrxn to Nlgs and LRRTMs requires extracellular $Ca2^{+}$.

While GluRδ2 is mainly expressed in cerebellar Purkinje cells, GluRδ1 is widely expressed in the developing forebrain including the caudate putamen and hippocampus. In a recent study it has been demonstrated that, in the presence of Cbln1 or Cbln2, GluRδ1 expressed in non-neuronal cells can induce inhibitory presynaptic differentiation on cultured cortical neurons by interacting with Nrxns containing the S4 insert [64].

## 4. Transient cell-cell interactions in neural development

*Trans*-synaptic adhesion molecules can affect the function of synapses at multiple levels, from recruiting synaptic proteins during synaptogenesis to regulating synaptic plasticity [1]. Thus, adhesion molecules are involved in dynamic processes such as synapse formation, which involves cell-type specific target recognition and synaptic plasticity, which requires the response to external stimulus or perturbations. A delicate balance between adhesion and de-adhesion cooperates generating robustness and flexibility to ensure normal nervous system development.




(A) GDNF induce *trans*-homophilic interactions between GFRα1 molecules, which are located at pre- and postsynaptic terminals. In the presence of GDNF, GFRα1, holds the terminals together triggering presynaptic maturation. This is mediated by GFRα1 and partially by the neural cell adhesion, NCAM, suggesting the involvement of an unknown partner (X). The role of GFRα1 in the postsynaptic terminal remains unknown (?).
(B) Cerebelin1 (Cbl1) acts as a bidirectional synaptic organizer by binding presynaptic β-Nrx (+S4) and postsynaptic GluRδ2 inducing pre- and postsynaptic differentiation.

**Figure 1.** Model describing ligand-induced *trans*-synaptic cell adhesion induced by (A) GDNF and (B) Cerebelin1.

The majority of *trans*-synaptic adhesion mechanisms known to date involve interactions triggered by the encounter between cell adhesion molecules inserted in the pre- and postsynaptic compartments. The activity of these cell-adhesion molecules may be regulated either developmentally or in response to neuronal activity. For instance, activity- dependent alternative splicing of Nrxn transcripts, or post-translational modifications such as glycosilation, polysialilation or palmitoylation drastically modify adhesive properties; binding partners and signaling properties of certain adhesion molecules [26,65,66]. Ligand-induced cell adhesion represents a previously unknown mechanism in which cell adhesion can be regulated not only by the presence of different forms of the associated receptor but also by ligand availability [56]. Furthermore, ligand secretion could also be regulated, for example by neuronal activity. Thus, *trans*-homophilic binding between GFRα1 molecules might be controled by the activity-dependent upregulation of GDNF, which has been shown to be upregulated by seizure activity [67,68]. On the other hand *trans*-heterophilic binding between GluRδ2 and Nrxn might also be regulated by the availability of Cbln1. It has been described that the expression of *Cbln1* mRNA is completely shut down in mature granule cells when the neuronal activity is increased by kainate [69]. Moreover, a recent study revealed that the-ligand binding domain of GluRδ2 can also bind to D-Ser inducing

conformational changes of the receptor that might modify the synaptogenic activity of the Nrnx/Cbl1/GluRδ2 complex [70,71] In addition, D-Ser has been shown to be regulated during development and also to be release from astrocytes in an activity-dependent manner [71]. Thus, the activity dependent regulation of each component of the Nrnx/Cbl1/GluRδ2 might increase the plasticity of the system.

Ligand-induced cell adhesion represents a new way to regulate intercellular interactions that may have broad implications not only for the development of the nervous system, but also in other tissues and organs.

## 5. Role of synaptic cell adhesion systems in nervous system disorders

The ability of *trans*-synaptic cell adhesion molecules to regulate synapse formation, maturation and plasticity supports the idea that deficits in many synaptogenic genes might be associated to neurodevelopmental and/or neuropsychiatric diseases.

Numerous studies indicate a genetic link of mutations in synaptic cell adhesion molecules to autism-spectrum disorders (ASD), in particular to Nlgs and Nrxns [72,73]. Mutations in genes encoding Nrxn1, Nlg3 and Nlg4 have been described to be associated with ASD. These alterations include different type of mutations that have been observed in a small fraction of patients. In particular thirteen different mutations have been described in *Nrxn1* gene: seven point mutations, two distinct translocations and four different deletions [72,74,75,76,77,78,79]; ten different mutations has been found in Nlg4 gene: two frameshifts, five missense mutations and three internal deletions; and a single mutation in *Nlg3* gene (R451C) [72,80,81,82,83]. Moreover, deletions in X-chromosome that includes the Nlg4 locus were detected in patients with autism [79,84,85,86]. Different studies have reported that Nlg4 deletions are also associated with other neurological disturbances including Tourette syndrome, attention deficit hyperactivity disorders, anxiety and depression. In addition, two different deletions of α-Nrxn1 have been observed in families with schizophrenia [58,87,88].

The role of some of these proteins in ASD has been validated in trasgenic animals. Thus, the Nlg3-R451C knockin mouse were reported to show a phenotype that shares some, but not all features with human ASD patients These mice show a modest impairment in social behaviour [89]. Moreover, Nlg-4 knockout mice show deficits in social interactions and communication [90].

In addition, members of SynCAM and LRRTM families have also been associated with nervous system disorders. SynCAM1 has been associated with ASD. Two missense mutations in the SynCAM1 gene of ASD patients and their families have been described. Interestingly, the mutations were located in one domain, which is essential for trans-synaptic interaction [91]. In a recent genetic study, polymorphisms in LRRTM3 were associated with ASD [88]. Moreover, LRRTM1 has been associated with schizophrenia [92].

There is no strong evidence connecting mutations in genes involved in ligand-induced cell adhesion systems with nervous system diseases. So far, only GluRδ1 was found to be associated with schizophrenia [92,93].

Further studies linking mutations in cell adhesion systems with nervous system diseases will contribute to the design of new diagnostic and therapeutic tools for these disorders.

## 6. Perspectives

It is well established, that cell-adhesion systems are in part responsible for the construction of neural circuits, synapse formation and plasticity. The correct function of the nervous system depends on the establishment of precise synaptic contacts between neurons and its specific targets, and deficits in genes coding for *trans*-adhesion molecules have been associated with learning deficits and cognitive impairments.

During the last years several adhesion molecules have been reported to participate in synapse development, including integrins, cadherins, protocadherins, NCAM, Neurexin-Neuroligin, LRRTM, SynCam, GDNF/GFRα, Nrxn-Cbl1-GluRδ2. The discovery of alternative *trans*-synaptic binding partners, in combination with their differential splice variants and isoforms, gave rise to a much larger spectrum of *trans*-synaptic interactions than was originally though. Furthermore, the mechanism of ligand induced cell adhesion considerably expands the functional repertoire of the ligands and receptors involved in these adhesion complexes and represents a new way to regulate *trans*-synaptic interactions that may have broad implications for the development of the vertebrate nervous system. Regulation of adhesion by soluble ligands allows a dynamic synaptic response to external stimulus during synapse formation and synaptic plasticity.

Based on this, the main challenge will be now to elucidate the complex code by which the *trans*-synaptic cell adhesion systems participate in the different steps of synapse formation, maturation and plasticity and to understand the importance of the different combinations of *trans*-synaptic partners in specific circuits. It will be important to understand how the combination of multiple synaptogenic systems may contribute to synaptic specificity controlling exactly where and when synapses form.

It will be also necessary to address whether individual synapse organizing protein instruct synaptic cell adhesion, or if the *trans*-synaptic interaction results from cooperation among different adhesion molecules. In some cases it is likely that the determination of initial synapse formation is mediated by multiple adhesion systems acting in parallel, as has been evidenced by $\alpha$-Nrxs and Nlgns knockout mice. The analysis of these animals showed that knockdown of these molecules, does not have consequence in synapse formation, suggesting that other adhesions systems compensate the deficiency in these mice [30,44].

The fact that multiple partners function at the same synapses opens the possibility that they cooperate in the recruitment of the same components to the synapse. Indeed, it has been described that overexpression of Nlgns and LRRTMs in primary hippocampal neurons cooperate in a synergistic manner in glutamate synapse development visualized by an increase in the recruitment of pre-synaptic proteins. Cooperation between different adhesion systems may help to stabilize interactions across the cleft by recruiting the pre- and postsynaptic machinery at multiple points. However the existence of mechanisms that

can modulate and modify these interactions should be important, especially for synaptic plasticity.

Further understanding of the molecular pathways and circuit events downstream these cell adhesion organizing systems will be extremely important in light of the role of *trans*-synaptic cell adhesion molecules in neurodevelopmental and cognitive diseases.

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