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Review

Cell adhesion and synaptogenesis

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Abstract: Synapses are inter-neuronal connections that are fundamental working units in neural networks. How synapses are molecularly constructed is a fascinating question, which attracted scientists' attention for many decades. Neuromuscular junction, a field pioneered by Te-Pei FENG and many others, has been an excellent model for studying synaptogenesis and paved the way for our understanding of the synapse formation in the central nervous system. Recent studies shed new light on the molecular mechanisms of central synapse formation by discovering a group of cell adhesion molecules exerting potent synaptogenic effects. This review will focus on those cell adhesion molecules which can induce central synapse formation when expressed in non-neuronal cells.

Key words: synaptogenesis; cell adhesion; synapse assembly

细胞黏附和突触发生

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摘 要：突触是神经网络中神经细胞间相互连接的基本工作单位。突触的分子构建是一个引人入胜的问题，数十年来一直吸引着科学家们的注意。冯德培和许多其他科学家早期在神经肌肉接头领域做出了开创性的研究工作。至今，神经肌肉接头仍是一个杰出的突触标本，为我们研究中枢神经系统的突触形成铺平了道路。近期的研究又有新的亮点，发现一组细胞黏附分子具有很强的突触发生作用，使中枢突触形成的分子机制更加明朗。本文综述了这些表达在非神经细胞里能引起中枢突触形成的细胞黏附分子的功能与特性。

关键词：突触发生；细胞黏附；突触组装
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1 Overview of synapse formation

Synapses are usually referred to junctions between two neurons in the nervous system. However, neuromuscular junction is also a special type of synapse formed between a neuron and a muscle cell, which has been extensively studied by Te-Pei FENG, Bernard Katz, and many other pioneers. Synapse formation is fundamental for brain circuit formation. However, for many decades, our understanding of central synapse formation has significantly lagged behind that of neuromuscular junction formation[1-3].

Synaptogenesis depends on crosstalk between presynaptic and postsynaptic elements. Recent studies have speeded up the progress in understanding the molecular mechanisms of glutamatergic synaptogenesis[4-8]. Using time-lapse imaging and retrospective immunocytochemistry, a time frame for new glutamatergic synapse formation has been proposed[9,10]. It was suggested that presynaptic proteins such as bassoon will appear in axon terminal within 30 min after initial axon-dendritic contact, and functional synaptic vesicle cycling can be detected within another 20 min of delay. In contrast, postsynaptic scaffold protein SAP90/PSD-95 and glutamate receptors will not be recruited to nascent axon-dendritic contacts until 60-90 min later. Consistent with the rapid function of presynaptic terminals, it was found that presynaptic vesicles

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can be pre-assembled with synaptic proteins into discrete packets and transport along developing axons[11]. Piccolo, one of the presynaptic active zone proteins, is transported to nascent synapses through dense core vesicles[10]. More importantly, the moving presynaptic packets may function as "orphan" release sites, capable of functional vesicle cycling without contacting postsynaptic dendrites[12]. Similarly, postsynaptic NMDA receptors have also been reported to form clusters and transport along dendrites before being recruited to nascent synapses[13]. Therefore, based on the studies of glutamatergic synapse assembly, a general consensus is formed that presynaptic differentiation precedes postsynaptic differentiation during central synapse formation[5,14]. However, our recent studies on GABAergic synaptogenesis suggest a different time course from that of glutamatergic synaptogenesis[15]. We found that when coculturing newly dissociated embryonic neurons with mature neurons, embryonic neurons are postsynaptically ready to receive GABAergic innervation but presynaptically incompetent to form synapses onto mature neurons. These results suggest that during GABAergic synaptogenesis, presynaptic differentiation may be the time-limiting step. Such rapid postsynaptic assembly during GABAergic synapse formation may also underlie the sequential formation of GABAergic and glutamatergic synapses during embryonic brain development[15,16]. One potential explanation for the rapid assembly of GABAergic postsynaptic apparatus is that it only requires $GABA_A$ receptors together with a single cell adhesion molecule (CAM) neuroligin-2 (NL-2)[17] (Fig.1). The rapid formation of GABAergic synapses may have an important functional significance because GABA plays an excitatory role during early brain development before switching to inhibitory function in the adult brain[18-20]. A proper temporal order of central synapse formation is critical for stereotypic construction of neural circuits during brain development.

Intercellular recognition mediated by CAMs plays an important role in helping axon and dendrites to find appropriate partners. Many CAMs have been suggested to be involved in synapse formation, synaptic transmission, and synaptic plasticity[21]. This review will focus on a subset of CAMs that have particular synaptogenic effects in inducing presynaptic or postsynaptic differentiation when expressed in non-neuronal cells (Fig.1).

2 Synaptogenic CAMs

2.1 Neuroligins and neurexins

2.1.1 Structure of neuroligins and neurexins

Neurexins are encoded by three genes in mammals, referred as neurexin I, II, and III. Each of the three genes has an upstream promoter to generate a longer α-neurexin and a downstream promoter to generate a shorter β-neurexin[22]. Neurexins are single transmembrane proteins with an N-terminal extracellular segment, a single transmembrane domain, and a short cytoplasmic tail. The extracellular segment of α-neurexins contains six LNS domains (laminin, neurexin, sex-hormone-binding protein

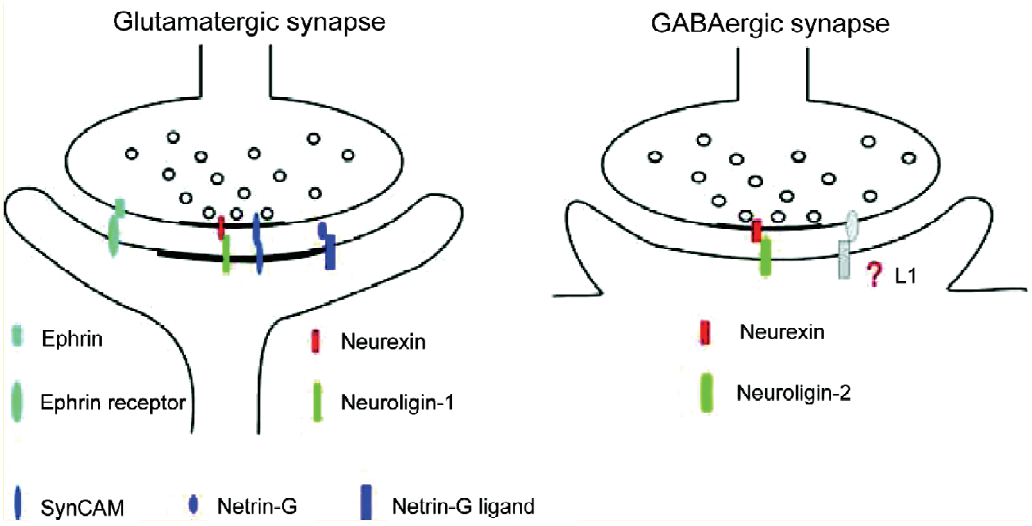

Fig. 1. Schematic illustration of trans-synaptic cell adhesion molecules that have been demonstrated to show synaptogenic effects when expressed in non-neuronal cells.

domain) organized with epidermal growth factor (EGF)-like domains into three homologous modules[23]. Alternative splicing occurs at five sites (named SS1-SS5) along the extracellular segment of α-neurexins. β-neurexins can be considered as N-terminally truncated version of neurexins since it has only a single LNS domain and the last two alternative splicing sites SS4 and SS5. The last LNS domain in both α- and β-neurexins is followed by a carbohydrate attachment sequence. The C-terminal of neurexin is conserved and contains a type II PDZ-binding motif.

Neuroligin-1 (NL-1) was first identified from brain lysates by affinity chromatography on neurexin 1α[24]. Four genes encoding neuroligin family members have been identified in rat and mouse, while five have been identified in the human genome[25,26]. The major extracellular domain of neuroligins is homologous to acetylcholinesterase (AChE), but lacks cholinesterase activities and binds to neurexins[27]. The AChE-like domain of neuroligins contains alternative splicing sites SSA and SSB[24,25]. A carbohydrate attachment region links the AChE-like domain to a single transmembrane region and the C-terminal cytoplasmic tail, which contains a type I PDZ-binding motif. The intracellular domains of neuroligins are less conserved comparing to the extracellular and transmembrane regions[25]. This indicates that the divergence of the cytoplasmic C-terminal may play a key role in the differential localizations and functions of different members of the neuroligin family.

Together, the cytoplasmic regions of both neurexins and neuroligins contain PDZ-binding domains, which are important for binding to other synaptic proteins. Furthermore, alternative splicing at the extracellular regions of both neurexins and neuroligins results in massive protein diversity. Such diversity may contribute to the selectivity of heteromeric binding between the pre- and postsynaptic sites, making it possible to form a variety of specific synapses.

2.1.2 Localization and interaction partners

At the cellular level, the expression patterns of the six major forms of neurexin (1α, 2α, 3α, 1β, 2β and 3β) broadly distribute in the brain and overlap with each other[28]. Neurexins are mainly localized at presynaptic nerve terminals[29,30], but recent study suggests that neurexins may also be localized at postsynaptic sites[31]. The mRNAs encoding NL-1, 2 and 3 have been detected in murine brain using *in situ* hybridization[24,25]. Subsequent studies showed that NL-1 expression is restricted to the central nervous system (CNS) and specifically localized at the postsynaptic membranes of excitatory synapses[32]. NL-2, on the other hand, is mainly found concentrating at inhibitory synapses[29,33]. The expression of NL-3 may not be restricted to neurons, but also found in glial cells[34].

The extracellular domains of presynaptic neurexins and postsynaptic neuroligins bind to each other to initiate synapse formation. The intracellular C-terminal of neurexins bind to synaptotagmin and PDZ domains of CASK, syntenin and Mint[35-38]. These interactions constitute a link from neurexins to both synaptic vesicles and the vesicle fusion apparatus. For neuroligins, they can bind to PSD-95 *in vitro*, a scaffold protein containing three PDZ domains that are capable of nucleate assembly of protein complexes at the postsynaptic membranes[39]. While it is not surprising that NL-1 interacts with PSD-95 at excitatory synapses[32,40], NL-2 at the postsynaptic membranes of inhibitory synapses does not have much opportunity to interact with PSD-95[33]. It has been found that NL-2 may interact with S-SCAM (synaptic scaffolding molecule) and associate with β-dystroglycan to form a protein complex but the functional significance has yet to be elucidated[41].

2.1.3 Functions of neuroligins and neurexins in synaptogenesis

2.1.3.1 Triggering pre- and postsynaptic differentiation

The pioneer work addressing the function of neuroligins in synapse development was done by Peter Scheiffele and colleagues using a coculture system of neurons with non-neuronal cells[42]. Their work showed that expression of neuroligin alone on the surface of non-neuronal cells is sufficient to invoke the accumulation of presynaptic protein synapsin in contacting axons and synaptic vesicles could undergo exocytosis. The function of neuroligins in triggering presynaptic development is mediated through its interaction with neurexin[42]. A subsequent study using neuroligin-coated beads further demonstrated that purified neuroligin can cluster neurexin and consequently trigger the recruitment of synaptic vesicles to the presynaptic membrane[30]. Conversely, neurexin alone presented on the surface of non-neuronal cells also induces both glutamatergic and GABAergic postsynaptic differentiation in contacting dendrites[29]. The effects of neurexin included the clustering of neurotransmitter receptors as well as postsynaptic scaffolding proteins such as PSD-95 at excitatory and gephyrin at inhibitory synapses. Electrophysiological recordings on non-neuronal cells expressing NL-1 together with glutamate receptors demonstrated that the artificial

synaptic contacts induced by NL-1 were indeed functional[43,44]. Similarly, functional GABAergic synapses were recently reconstituted through coexpression of NL-2 and $GABA_A$ receptors in HEK cells that were cocultured with neurons[17]. Importantly, the heterologous synapses formed between neurons and HEK cells not only showed robust spontaneous synaptic responses, but also were capable of action potential-evoked fast neurotransmission, indicating that these synapses are fully functional[17].

The synaptogenic activity of neuroligins in heterologous expression systems has been directly confirmed in cultured neurons. Overexpression of NL-1, 2 and 3 in neurons was found to increase the number of presynaptic terminals contacting the transfected neurons[45], while knockdown of neuroligins using shRNAs resulted in a loss of synapses[46]. Furthermore, the effect of neuroligin overexpression can be blocked by a fusion protein containing the extracellular domain of neurexin-1β[45], indicating the importance of an endogenous interaction between neuroligins and neurexins. Live fluorescence imaging revealed that NL-1 can form postsynaptic protein complex with PSD-95, and the sites containing such complexes can transform opposing presynaptic sites into functional nerve terminals[47]. These results suggest that neuroligin-containing postsynaptic complexes can serve as predetermined hotspots for the establishment of new functional synapses.

2.1.3.2 Modulation of excitatory *versus* inhibitory synapse development

Interplay between neuroligins and PSD-95 may participate in regulating the balance between excitatory and inhibitory synapses. When PSD-95 and NL-1 are coexpressed in hippocampal neurons, the excitatory synapses were enhanced but inhibitory synapses were reduced, tilting the ratio of excitatory to inhibitory synapses[48]. Overexpression of PSD-95 also resulted in a shift in the distribution of NL-2 from inhibitory to excitatory synapses[45]. Electrophysiological recordings showed that PSD-95 overexpression increased the ratio of excitatory to inhibitory synaptic currents, while knockdown of PSD-95 through RNA interference (RNAi) reduced the ratio of excitatory to inhibitory synaptic currents[48]. Furthermore, downregulation of NL-1, 2 and 3 expression using RNAi led to a predominant reduction of inhibitory synaptic function, which altered the functional balance between excitatory and inhibitory synapses in hippocampal neurons[46]. An alternative explanation for this result could be that inhibitory synapse formation is mainly determined by the expression level of NL-2, but excitatory synapses can be formed in the absence of NL-1 and 3, such as by the other CAMs discussed below.

Alternative splicing provides another mechanism through which neuroligins selectively affect the development of excitatory *versus* inhibitory synapses. The extracellular splice insertions (site A and site B) in NL-1 and 2 may restrict their localization to excitatory and inhibitory synapses through interacting with corresponding presynaptic neurexins[49]. The neuroligin isoforms with A insertion, predominantly NL-2A, showed a selective synapse-inducing activity on GABAergic synapses. In contrast, the presence of B insertion in NL-1 variants NL-1B and NL-1AB restricts their functions at glutamatergic synapses. These findings are consistent with another work from Sudhof's group that the splice site B of neuroligins determines the interaction with neurexins[50].

2.1.3.3 Maintenance and maturation of synapses

The neurexin-neuroligin interaction may induce initial formation of silent synapses in hippocampal neurons. In particular, neurexin expressed in non-neuronal cells clusters PSD-95 through neuroligin, and thus induces synapses containing only NMDA receptors but not AMPA receptors[51]. The insertion of AMPA receptors into the induced contacts requires NMDA receptor activation and Ca^{2+}/calmodulin-dependent kinase II (CaMKII). The generation of neuroligin knockout mice provided a powerful tool for understanding the function of neuroligins *in vivo*[52]. NL-1, 2 and 3 single, double and triple knockout mice were generated recently, but deletion of all the three neuroligins did not affect the total number of synapses, as shown with immunostaining and electron microscopy. However, the NL-1, 2 and 3 triple knockout mice showed a dramatic decrease in inhibitory and a moderate reduction in excitatory synaptic transmission in the brain stem respiratory center. These results suggested that proper neuroligin function is important for maintaining an appropriate ratio of excitatory to inhibitory synaptic currents in the brain network, while compensatory mechanisms may exist in establishing initial synaptic contacts. A subsequent work further revealed the mechanism through which neuroligins function in specifying and validating synapses[53]. It was suggested that the excitatory synapse-boosting activity of NL-1 is dependent on the activation of NMDA receptors and CaMKII, whereas the effect of NL-2 in inhibitory synapse-boosting requires the general synaptic activity[53].

The neuroligin-neurexin signaling was also shown to regulate the short-term plasticity by altering the presynaptic release probability[40]. It was demonstrated that overexpression of postsynaptic NL-1 or PSD-95 increased the presynaptic sensitivity to extracellular Ca^{2+}, which consequently raised the release probability. A recent work revealed a novel function of neurexin in modulating synaptic contact. Neurexins are found not only localized to axons and presynaptic terminals, but also abundant in the postsynaptic density[31]. The postsynaptically expressed neurexin-1β directly interacts with NL-1 and blocks the synaptogenic activity of NL-1, indicating a possible function of postsynaptic neurexins in silencing the activity of a synaptic adhesion complex and thus modulating the synapse maturation after the initial formation.

2.2 SynCAM

SynCAMs are transmembrane proteins that belong to the superfamily of immunoglobulin (Ig) CAMs. SynCAM family consists of four members, SynCAM1, 2, 3, 4, all of which share similar domain structure[54]. SynCAMs contain three extracellular Ig-like domains in the N-terminal, a single transmembrane region, and a short cytosolic domain. The cytoplasmic carboxy terminal of the SynCAM proteins includes a PDZ-binding domain that binds to PDZ-domain proteins CASK and syntenin[55]. The highly conserved C-terminal also contains protein 4.1 motif that is implicated in cytoskeleton organization[54]. All four isoforms of SynCAMs undergo alternative splicing and the genes encoding the family members are only found in vertebrates. SynCAM proteins show a Ca^{2+}-independent homophilic cell adhesion activity and the expression patterns of family members show variation in terms of tissue distribution as well as developmental stages.

So far, SynCAM1 has been the most investigated member among SynCAM family. SynCAM1 was initially identified as a human tumor suppressor gene and the defects in this gene have been found to promote metastasis of lung carcinoma[56]. SynCAM1 was also reported as an important regulator of spermatogenesis[57] and cell adhesion in epithelial cells[58]. Notably, SynCAM1 was recently reported as a synaptic CAM, critical for excitatory synapse formation. Expression of SynCAM1 along with AMPA receptor subunit GluR2 in HEK 293T cells was found to be sufficient to induce postsynaptic responses in non-neuronal cells when co-cultured with hippocampal neurons[55]. Further investigation of the synaptogenic effects of SynCAM1 and NL-1 yielded interesting differential roles in excitatory synapse formation[44]. Overexpression of SynCAM1 in cultured hippocampal neurons increased the postsynaptic responses, whereas overexpression of NL-1 increased synapse number and spine density without changes in synaptic responses. These observations led to a speculation that NL-1 leads to activity-independent formation of *de novo* synapses and spines, whereas SynCAM1 increases activity-dependent synaptic efficiency in immature neurons. Another important result of this study was that SynCAM1 preferentially promotes excitatory but not inhibitory synapse formation[44].

SynCAM3 and SynCAM4 are initially isolated as tumor suppressor genes as well[59]. Particularly, SynCAM3 has been shown to be brain-specific and localized at contact sites of axon terminals and glial cells, suggesting a role in glial processing[60]. SynCAM4, on the other hand, has been found as the predominant isoform expressed in dorsal root ganglion Schwann cells where it functions as the glial binding partner for axonal SynCAM3[61]. The interaction between SynCAM4 and SynCAM3 mediates axon-glial adhesion and more importantly, is required for myelination. Taken together, increasing evidence suggests a much broader role for SynCAM family proteins in neuronal development not only in the CNS but also in the peripheral nervous system (PNS). Future studies should elucidate whether SynCAM2, 3, and 4 are involved in excitatory and/or inhibitory synapse formation.

2.3 Ephrin receptors and ephrin ligands

The ephrin receptor family is the largest family of receptor tyrosine kinases composed of at least 14 known members in the vertebrate genome. Ephrin receptors are divided into two subfamilies: EphA (EphA1-A8, A10) and EphB (EphB1-B4, EphB6)[62]. Ephrin ligands also belong to a large family of proteins comprised of two subfamilies, ephrin A (ephrin A1-A5) and ephrin B (ephrin B1-B3). Ephrin receptors and ephrin ligands are transmembrane proteins that bind through their extracellular N-terminal domain[63]. Ephrin receptors are further characterized by a cysteine-rich motif and two fibronectin type III repeats on the extracellular side as well as a highly conserved, multifunctional C-terminal domain[63]. Ephrin receptor C-terminal has four segments: the juxtamembrane domain bearing the conserved tyrosine residues, tyrosine kinase domain, sterile α-motif and a PDZ-binding motif. According to the classical model, ephrin As and ephrin Bs bind to EphA receptors and EphB receptors, respectively. However, there is a large variation in affinity within each subgroup and binding

between EphA and ephrin Bs and *vice versa* has also been reported[64,65]. Transcellular interaction of ephrin ligands and ephrin receptors can generate bidirectional signals, implicated in an array of functions including axon outgrowth and guidance[66,67], cell migration[68], and synaptic plasticity[69] (reviewed in[63,65]). Through its cytoplasmic PDZ-binding domain, ephrins recruit PDZ-domain proteins such as glutamate-receptor-interacting-protein-1 (GRIP-1), syntenin, and protein kinase-interacting protein Pick1[70].

Ephrins and ephrin receptors are expressed throughout the developing and mature nervous system, including regions with high plasticity where ephrin receptors localize postsynaptically as opposed to presynaptic ephrin ligands[71]. The extracellular domain of EphB receptors may directly interact with the extracellular region of NMDA receptors[72]. Ephrin B2 activation of EphB receptors has been shown to increase phosphorylation of NMDA receptors through Src-tyrosine kinase activity, which then enhances glutamate-induced Ca^{2+} influx through NMDA receptors[73]. EphB2 receptors have also been found to be colocalized with AMPA type glutamate receptors in dendrites, and that ephrin B2 stimulation increases surface expression of GluR2/3 subunits of AMPA receptors[74]. Remarkably, when expressed in HEK 293T cells, EphB2 receptors can induce clustering of presynaptic vesicles in contacting axons to trigger presynaptic differentiation[74]. Formation of new synapses is presumably through the trans-synaptic interaction between EphB2 receptors in non-neuronal cells that act as postsynaptic compartments and ephrin ligands in neuronal axons that serve as presynaptic partners. Moreover, reducing the expression of EphB2 receptors in cortical neurons by siRNA knockdown method causes a significant decrease in spontaneous postsynaptic responses and in the number of excitatory synaptic specializations[74]. Neurons from triple knockout mice ($EphB1^{-/-}2^{-/-}3^{-/-}$) show specific deficiencies in excitatory but not inhibitory synapse formation, as judged by immunostaining for excitatory and inhibitory synaptic markers[75]. Although EphB receptors are critical for spine development and synapse formation, the triple knockout mice are viable and able to breed, implicating that their synaptogenic functions can be largely compensated by other molecules such as NL-1 and SynCAM1 in the CNS.

2.4 Netrin-G ligand (NGL)

An additional inducer of excitatory synapse formation is the NGL family. Netrin-related molecules, netrin-G1[76] and netrin-G2[77], have been identified as secreted proteins localized at plasma membranes through glycosyl phosphatidylinositol (GPI) lipid anchors. Netrin-G1 and netrin-G2 lack affinity for netrin receptors and are evolutionarily distinct from the netrin family. Netrin-G family members show non-overlapping but complimentary expression patterns in the brain. Netrin-G1 is largely distributed in the thalamus, olfactory bulb, and inferior colliculus, while netrin-G2 is found in the cerebral cortex and superior colliculus[76,77]. Netrin-G1 and netrin-G2 are implicated in axon and dendrite outgrowth as well as excitatory synapse formation through interactions with members of recently identified NGL family. Note that netrin-Gs are ligands and NGLs are receptors for netrin-Gs. NGL family has three known members: NGL-1[78], NGL-2, and NGL-3[79]. NGL-1 and NGL-2 directly interact with netrin-G1 and netrin-G2, respectively, in an isoform-specific and Ca^{2+}-independent manner. NGL-1 and NGL-2 show diverse but complementary distribution patterns throughout the brain, which reflect that of their binding partners. NGL-3, on the other hand, does not bind to netrin-G1 or netrin-G2 and is expressed throughout the brain[79]. Extracellularly, NGLs consist of nine leucine-rich repeat (LRR) domains flanked by N-terminal LRR and C-terminal LRR, followed by an Ig domain. LRR motifs have been implicated in NGL-netrin interaction. The PDZ-binding motif located on the cytoplasmic tail suggests that NGLs have a role in organizing synaptic components.

The interaction between netrin-G1 and NGL-1 in thalamocortical axons is shown to be critical in promoting neurite outgrowth, an effect partially mediated by the GPI anchor of netrin-G1[78]. NGL-2, on the other hand, has been recently identified as a binding partner of netrin-G2 in a complex that includes PSD-95 and NMDA receptors[79]. Importantly, when expressed in non-neuronal cells or fused with beads, NGL-2 is capable to induce functional presynaptic specialization in cocultured neurons[79]. Decreasing NGL-2 expression or NGL-2 adhesion in neurons reduced the number of excitatory but not inhibitory synapses, suggesting that NGL-2 regulates the formation of excitatory synapses through trans-synaptic adhesion[79]. Recently, through a loss-of-function study it is shown that netrin-G1 and netrin-G2 are selectively localized to the axons of distinct pathways, where they interact trans-synaptically with dendritic NGL-1 and NGL-2, respectively[80]. The finding that netrin-Gs and their receptors NGLs interact trans-synaptically might be supplemental to the synaptogenic functions of neuroligins, SynCAM, and ephrin-ephrin receptors.

2.5 L1 family

L1 family of CAMs is a subfamily of Ig superfamily that has four members in the vertebrate genome: L1-CAM, neurofascin, Nr-CAM, and CHL1[81]. Each member of the L1 family contains a large extracellular domain with six Ig-like domains and five fibronectin III-like domains, a transmembrane segment, and a highly conserved cytoplasmic domain. The cytoplasmic domains of most L1-type proteins include ankyrin (FIGQY) and ezrin-radixin-moesin (ERM)-binding motifs, which serve as the links to spectrin and cytoskeleton to mediate membrane-cytoskeleton connections[82,83]. Previous data suggest that L1 proteins play a role in the development of both CNS and PNS. L1 family is implicated in myelination[84], axon guidance and bundling[85-88], and long-term synaptic plasticity[89,90]. Notably, mutations in the L1 have been identified in a variety of neurological disorders including X-linked hydroencephalus and MASA syndrome (mental retardation, aphasia, and shuffling gait)[91,92].

Recently, L1 family of proteins emerged as important players in synapse formation. First, postsynaptic neurofascin was found to be a major molecular cue to direct GABAergic innervations from basket cell axon terminals to Purkinje cell axon initial segment[93]. Another member of the family, L1-CAM, also called L1, is found essential in organizing nicotinic synapses in both neurons and their target muscle cells[94]. L1 localizes both pre- and post-synaptically and the homophilic interaction of L1 can induce alignment of pre- and postsynaptic specialization. Interestingly, expressing L1 in HEK 293T cells can induce nicotinic presynaptic differentiation in cocultured chick ganglion neurons as assessed by immunostaining for synaptic markers[94]. Moreover, L1 regulates the proper organization of both pre- and postsynaptic elements at neuromuscular junctions *in vivo*.

3 Conclusion

We have summarized here 5 sets of CAMs that can mediate trans-synaptic adhesion and are capable of triggering pre- or postsynaptic differentiation when expressed in non-neuronal cells. Interestingly, 4 of them, including NL-1, SynCAM1, EphB2 receptor, and NGL-2, are found to promote specifically excitatory glutamatergic synaptogenesis. In contrast, only NL-2 so far has been found to promote exclusively inhibitory GABAergic synaptogenesis. L1 promotes cholinergic synapse formation, but whether it can trigger *de novo* GABAergic synapse formation when expressed in non-neuronal cells remains to be investigated. The neuroligin triple knockout neurons showed a dramatic reduction in inhibitory synaptic functions but milder defect in excitatory functions, confirming that excitatory synaptogenesis is promoted by multiple, possibly redundant, synaptogenic molecules during brain development. How these different synaptogenic molecules work synergistically to ensure proper neural circuit formation remains an open field for future studies. An alternative CAM other than NL-2 that can promote inhibitory synapse formation is also awaiting for further exploration.

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