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EXTRACELLULAR MATRIX MOLECULES AND FORMATION OF CNS SYNAPSES

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1. SUMMARY

Intracellular scaffolding proteins play important roles in synaptogenesis by linking major components of pre- and postsynaptic machineries with pre- and postsynaptic cell adhesion molecules. Molecules of the extracellular matrix (ECM), secreted from neurons and glial cells, also form scaffolds, although in the extracellular space. These scaffolds are involved in recruitment and stabilization of synaptic components. For instance, ECM molecules may cluster postsynaptic glutamate receptors via direct interactions with the extracellular domains of these receptors in a synapse-specific manner, or accumulate presynaptic Ca^{2+} channels and growth factors. Furthermore, binding of ECM molecules to cell surface receptors, such as integrins and apolipoprotein E receptor 2, may trigger intracellular signaling cascades resulting in maturation and plasticity of synapses. Additionally, thrombospondins (TSPs) and tenascin-R have prominent effects on formation of synapses via not-yet-identified mechanisms. In this chapter we review available data on synaptogenic activities of ECM molecules, suggesting that these molecules are important for many aspects of synaptic function in the central nervous system (CNS).

2. INTRODUCTION

Tremendous advances have been made in our understanding of the proteins and signals required for synaptogenesis in the CNS. An array of both intrinsic neuronal proteins and extrinsic signals from other cells – such as glia – influence

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aspects of CNS synapse formation and function. An emerging picture is now forming of which of these proteins and signals are necessary and sufficient for synaptogenesis. Thus, it appears that transmembrane proteins such as the neurexin–neuroligin proteins and SynCAM are sufficient to induce formation of CNS synapses[1–3] (Chapters 4,7,8). However, these signals do not act alone. Aspects of synapse formation, stabilization, elimination, and plasticity are influenced by other signals that are either intrinsic or extrinsic to neurons. For example, cadherins, ephrins, neuroligan, Wnts, TGFβ family members, neuronal pentraxins, FGF-22, and the ECM molecules have been identified as helping to promote pre- or postsynaptic differentiation in a variety of invertebrate and vertebrate preparations[2,4–7]. Here, we focus on the role of the ECM in aspects of CNS synapse formation and stabilization. Several recent reviews have provided insight into the ECM's role in synaptic plasticity[8,9]. While ECM molecules have an established and important role in PNS synaptogenesis, the function of ECM in CNS synaptogenesis is relatively poorly understood. Nevertheless, several interesting studies have recently implicated ECM in different aspects of CNS synapse formation and function.

3. THROMBOSPONDINS

3.1. The Thrombospondin Family

TSPs form a highly conserved family of molecules[10] and constitute a gene family of five members in vertebrates. All TSPs are extracellular multimeric, multidomain calcium-binding glycoproteins that function at cell surfaces and in the ECM[10–13]. Their best known functions are to promote cell attachment and to regulate the cytoskeleton, migration, and angiogenesis. Subgroup A TSPs (TSP1 and TSP2) are homotrimeric, whereas subgroup B TSPs (TSPs 3, 4, and 5) are homopentameric molecules. Members of each subgroup are very closely related. TSP5 is only found in cartilage, but there is evidence that the other four forms are expressed at varying times during development in the nervous system. TSP1 is the best studied of the five TSPs. Structurally, TSP1 consists of a heparin-binding N-terminal domain (HBD), a linker with homology to procollagen, three TSP-type-1 (properdin) repeats, three TSP-type-2 (EGF) repeats, seven TSP-type-3 (calcium binding) repeats, and a cell-binding carboxyl-terminal domain (CBD) (Figure 11.1). Its C-terminal domain is associated with adhesive functions, whereas its amino-terminal domain is implicated in de-adhesive functions. TSP2 has a very similar structure. TSP3, 4, and 5 lack the N-terminal domains, procollagen homology linker, and the TSP-type-1 repeats, and display much more restricted expression patterns. Importantly, each of the TSP domains binds to different receptors.

In the nervous system, TSP1 and TSP2 have been found to be expressed by astrocytes only during postnatal development[14,15]. TSP3 has been observed predominantly in embryonic brain. TSP4 is expressed by CNS neurons in adulthood rather than during postnatal development[16] and is highly concentrated in synaptic layers in the adult retina and brain, as well as at the mature NMJ. The functions of these TSP isoforms in the nervous system are not known.

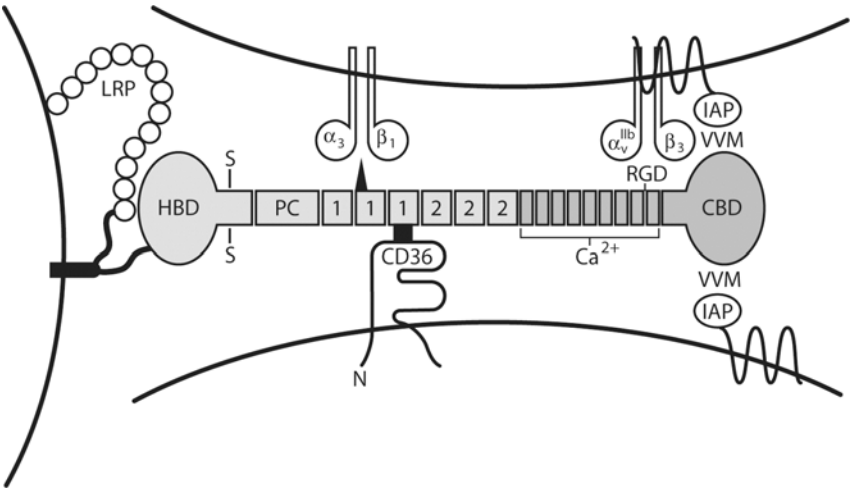

Figure 11.1. Structure of TSP1 and 2. The molecules comprise distinct domains that in non-neuronal cells have been found to bind to distinct receptors and mediate different functions of the proteins. TSP1 and 2 consist of a heparin-binding N-terminal domain (HBD), a linker with homology to procollagen, three TSP-type-1 (properdin) repeats (labeled as 1), three TSP- type-2 (EGF) repeats (labeled as 2), seven TSP type-3 (Ca^{2+} binding) repeats, and a cell-binding carboxyl-terminal domain (CBD). VVM-containing motifs in the CBD of TSP bind to integrin-associated protein (IAP). The HBD in involved in interaction with low-density lipoprotein receptor-related protein (LRP) and other proteins (see ref. 10).

3.2. Thrombospondin Receptors Are Highly Localized to CNS Synapses

Among the TSP receptors that are highly localized to synapses in the CNS are multiple integrins and integrin-associated protein (IAP/CD47). Integrins comprise a large family of cell adhesion molecules that mediate interactions between the extracellular environment and the cytoplasm[17–19]. Integrins are expressed as cell surface heterodimers consisting of α and β subunits. There are 16 different α and eight different β mammalian integrin subunits, which associate to form 22 recognized α β heterodimers. Each integrin recognizes specific ligands, often ECM molecules such as laminin and fibronectin, or other cell surface receptors such as cell adhesion molecules. Recently, it has been found that integrins are localized to synapses, where they participate in synaptic development, function, and plasticity[20–24]. CD47/IAP is also highly localized to synaptic regions throughout the brain and retina[25,26], where its function is unknown.

3.3. Induction of CNS Synapses by Thrombospondins

Thrombospondin has recently been identified as a component of the CNS ECM that is both necessary and sufficient to form synapses *in vitro* and plays a role in synapse formation *in vivo*. Using purified cultures of neurons and glia, numerous

studies have found that neurons in the absence of other cell types can survive and extend robust processes, but they do not form many synapses. Interestingly, another cell type associated with synaptic structures *in vivo*, the astrocytes, produces soluble signals that can increase the number of synapses on neurons nearly 10-fold[27–29]. This increase in synapse number is seen when media conditioned over a feeding layer of astrocytes (astrocyte conditioned media) is added to neurons, indicating that the signal is secreted from astrocytes, much as ECM molecules are secreted. What is the identity of this astrocyte-derived synapse promoting factor(s)? One astrocyte-derived synapse promoting signal turns out to be TSP.

Evidence that TSP is the astrocyte-derived signal that increases synapses on purified neurons comes from the recent work of Christopherson and co-workers[30]. These authors found that astrocytes *in vivo* and *in vitro* express high levels of TSP1 and TSP2 mRNA. Interestingly, *in vitro*, TSP2 seems to be the main protein expressed. Is TSP2 necessary and sufficient to increase the synapse number *in vitro*? Addition of purified recombinant TSP2 to cultures of neurons resulted in a dramatic increase in synapse number, similar to the effects of astrocyte-conditioned media[30], indicating that TSP2 is indeed sufficient to increase synapse number (Figure 11.2A; Colorplate 8). To determine if TSP2 is a necessary signal from astrocytes for their synapse-promoting ability, TSP2 was removed from astrocyte-conditioned media with a TSP2-specific antibody. TSP2 depletion almost entirely eliminated the synapse-inducing activity of astrocyte-conditioned media. These findings taken together indicate that TSP2 is sufficient and necessary for astrocyte-conditioned media to induce synaptogenesis between retinal ganglion cells *in vitro*. TSP1 had a similar synapse-promoting effect as TSP2 and appeared to be expressed *in vivo*[30]. Therefore, it is likely that both TSPs play a role in synaptogenesis in vivo. To determine what role TSP1 and TSP2 play in synaptogenesis *in vivo*, the recently generated TSP1 and TSP2 double knockout (KO) mice were analyzed.

3.4. TSP1 and TSP2 Double Knockout Mice Have A Reduced Number of Synapses

Analysis of double KO brains found a 30% decrease in synapse number at early postnatal ages (P5–6) and this reduction in synapses is maintained into adulthood[30]. Interestingly, no significant effect on synapse number was seen in the TSP1 or TSP2 single KOs. This indicates that both TSP1 and TSP2 are required *in vivo* for normal synapse formation or stabilization. Because of the high homology of the TSP1 and TSP2 orthologs, it is likely that they can serve redundant functions in the nervous system, each being able to regulate synaptogenesis.

How do TSP1 and TSP2 regulate synaptogenesis *in vitro* and *in vivo*? One interesting property of TSP is its ability to regulate function of other adhesion molecules. This raises the possibility that TSP functions by regulating the adhesiveness or signaling of other proteins already implicated in synapse formation, such as neurexin–neuroligin or SynCAM. Whether TSP1 and TSP2 function by regulating such molecules and how these TSPs send signals to neurons has yet to be determined.

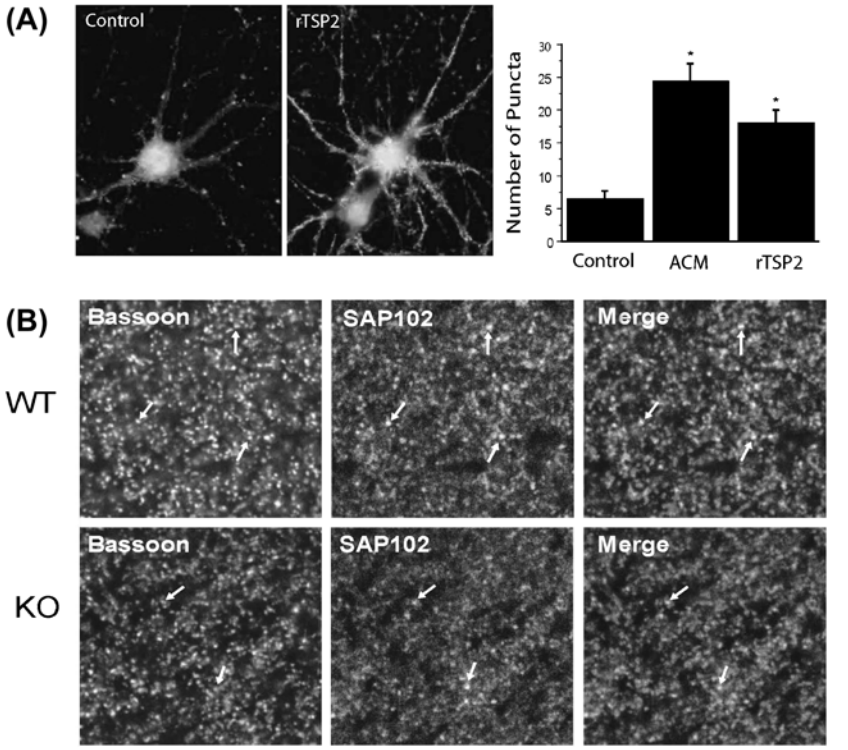

Figure 11.2. TSP is Necessary and Sufficient for Synapse Formation. (A) Purified CNS neurons do not form synapses under control condition (Control) as seen by the absence of synaptic puncta after staining for presynaptic synaptotagmin and postsynaptic PSD-95. Addition of recombinant TSP2 (rTSP2) induces a dramatic increase in the number of colocalized presynaptic puncta and postsynaptic puncta (yellow puncta in Colorplate 8). Graph shows a nearly 4-fold increase in synapse number after addition of rTSP2 that is comparable to the increase elicited by the astrocyte-conditioned medium (ACM). (B) Postnatal day 21 wild-type (WT) and TSP1/2 double knockout (KO) mice brains were sectioned and stained for presynaptic marker Bassoon and postsynaptic marker SAP102. KO animals show a reduction in the number of colocalized synaptic puncta (arrows). Reprinted from ref. 30, Copyright (2005) with permission of Elsevier.

4. NEURONAL PENTRAXINS

4.1. The Pentraxin Family

The pentraxin family is a large family of proteins defined by a conserved motif, the pentraxin domain. Some pentraxins are structurally characterized by the arrangement of subunits in a pentamer. The pentraxin family is further broken up into two main types of pentraxins, the short pentraxins and the long pentraxins. The short pentraxins consist of C-reactive protein (CRP) and serum amyloid P. The long pentraxins consist of pentraxin 3 and 4 (PTX3 and PTX4), neuronal pentraxin 1 and 2 (NP1 and NP2, also known as Narp), and neuronal pentraxin receptor (NPR)[31].

The first identified role for pentraxins was a role in innate immunity. Innate immunity is characterized by the recognition of pathogens and damaged tissue by pattern recognition receptors. The first pentraxin to be identified, CRP, is an example of such a receptor. This pentraxin binds to the *C*-polysaccharide of *Streptococcus pneumoniae* and mediates the immune attack and kills sequence for complement-mediated lysis of these pathogenic bacteria. Similarly, serum amyloid protein also binds a variety of pathogens and mediates the innate immune response to pathogens. In addition, pentraxins appear to play an important role in tissue remodeling associated with fertility and inflammation[32–34]. The neuronal pentraxins, NP1[35], NP2[36–39], and NPR[40], were identified as proteins that bind to affinity columns of the presynaptic snake venom toxin, taipoxin, and/or the lumenal calcium-binding protein TCBP49[41]. NP1, NP2, and NPR protein sequences are 50% identical to each other and their carboxy terminal half is 20–30% identical to classical pentraxins such as the acute phase proteins, CRP[42], serum amyloid P protein[43], and the long pentraxin, PTX3[44]. Neuronal pentraxins form heteromultimers[45]. While NP1 and NP2 are secreted when expressed alone, they can also be tethered to the cell membrane when expressed as heteromultimers with NPR. NP2 has also been identified as a synaptic protein that is rapidly and dramatically upregulated by neuronal activity[39]. This places NP2 in the family of immediate early genes. The function of NP1 and NP2 *in vivo* is unknown, although both NPs have been implicated in regulating glutamate receptor clustering *in vitro*.

4.2. Pentraxin's Role in Glutamate Receptor Clustering

One of the best studied functions of NPs is the clustering of AMPA receptors (AMPARs). Both NP1 and NP2 are capable of clustering AMPARs *in vitro*. Initially, NP2 was found to cluster AMPARs in culture[46]. This study reported that NP2 was enriched at excitatory synapses both on hippocampal and spinal neuron cultures, and the expressed Myc-tagged NP2 was delivered to synapses. Strikingly, NP2 molecules co-clustered and co-immunoprecipitated with AMPA receptor subunits GluR1–3, but not with the AMPA receptor subunit GluR4, NMDA receptor subunits NR1 and NR1/2A, or kainate receptor subunit GluR6. Expression of a dominant-negative NP2 mutant protein, which prevented secretion of endogenous NP2, inhibited NP2 accumulation at synapses and affected clustering of AMPARs. Because it is not entirely clear that the dominant-negative NP2 exerts its effects only through NP2 secretion, these results must be interpreted somewhat cautiously. It is possible that endogenous levels of NP2 are not directly involved in AMPAR clustering, but this work certainly implies that NP2 plays some role in this process.

Recent work has suggested that NP2 may also indirectly cluster NMDA-type receptors in spinal neurons. Mi and co-workers[47] found that NP2 may act through the AMPAR subunit GluR2 to cluster NMDARs containing the NR2A or NR2B subunits. This clustering depended both on the GluR2 C-terminal tail and on the C-terminal tail of stargazin, a protein found to cluster AMPARs at synapses[48]. NP2 does not directly bind to either NMDARs or stargazin, implying that there may be an interaction between clustered AMPARs and NMDARs through stargazin in

nonspiny neurons. Interestingly, these authors also found that stargazin DC (Stargazin without the C-terminal domain) does not affect surface GluR1 clusters in transfected nonspiny motor neurons and hippocampal interneurons, despite having a potent effect on reducing GluR1 clusters in spiny hippocampal pyramidal neurons[48]. Because NP2 can cluster AMPARs on these types of neurons, one interpretation of this result is that NP2 takes the place of stargazin for AMPAR clustering. However, stargazin is one member of a family of proteins termed Transmembrane AMPA Receptor Proteins (TARPs) with AMPAR binding and surface expression and synaptic targeting functions[49]. Thus, it is quite possible that different members of the TARP family are required for AMPAR clustering in different types of synaptic connections. Nevertheless, based on the previous work, it appears that NPs play a role in the clustering and synaptic distribution of AMPARs, especially at nonspiny synapses. Because NP1 shares about 50% sequence identity with NP2 and has also been shown to cluster AMPARs, it is thought that these two proteins may function in concert to cluster AMAPRs at nonspiny synapses[50–52].

4.3. Pentraxin's Role in Synaptogenesis

Overexpression of NP2 not only induced clustering of AMPARs but also increased the number of excitatory synapses in hippocampal cultures[46]. Furthermore, NP2 expression in transfected HEK293 cells was sufficient to induce AMPAR clustering on neuronal dendrites that contacted the transfected cells. Interestingly, these overexpression studies revealed that NP2 can be secreted to synapses both from the presynaptic terminal as well as from the postsynaptic dendrite, although it is not clear whether under normal conditions there is significant dendritic expression of NP2.

In subsequent studies it was found that dominant-negative NP2 not only reduced the number of GluR1 subunit AMPAR clusters at contact sites between a dominant-negative NP2 expressing neuron and a postsynaptic neuron, but also induced a slight, although significant, decrease in the number of excitatory synaptic contacts in this connection[50]. No effect was found on the number of inhibitory synapses as assessed by gephyrin or GAD clusters. These studies led to a hypothesis that NP2 in the CNS may act analogously to agrin clustering of nicotinic acetylcholine receptor clustering in the PNS (see Chapter 1), being necessary and sufficient to cluster AMPARs at excitatory synapses and thereby leading to increases in the number of synapses found in culture. Both NP1 and NP2 are able to cluster AMPARs, but pentamers comprising both NP1 and NP2 have greater clustering activity than pentamers of either alone[52]. Interestingly, while secretion of NP2 is activity dependent, secretion of NP1 is not[39,52]. This finding leads to a model in which neurons have a low level of receptor clustering activity through NP1 that is dramatically upregulated by activity and subsequent release of NP2. Thus, this mechanism appears to contribute to both activity-independent and activity-dependent excitatory synaptogenesis[52]. What roles NP1 and NP2 play *in vivo* are completely unknown. NP1, NP2, and NP1/2 double KO mice must be carefully analyzed to understand the role of this neuronal ECM protein in synapse formation and function.

5. TENASCIN-R

5.1 The Tenascin Family

Tenascins are multimeric ECM glycoproteins. In vertebrates, five tenascins are found in various tissues, tenascins-C, -N, -R, -X, and -W. Of these, tenascins-C and -R are not only found in the CNS but also implicated in modulating different aspects of synaptic plasticity[8,53]. Structurally, tenascins-C and -R contain EGF-like repeats, fibronectin type III repeats, and a fibrinogen domain. Tenascin-R is one of major components of the perineuronal nets that surround a subset of neurons in the brain and spinal cord[54]. These nets – known already to Golgi and Cajal – have recently received renewed interest because of several studies suggesting that they may have inhibitory modulatory effects on regeneration and structural plasticity[55,56].

5.2. Role of Tenascin-R in Formation of GABAergic Synapses

Electron microscopic analysis of tenascin-R KO mice revealed a reduction in the number of inhibitory somatic synapses on CA1 pyramidal neurons. In addition, existing synapses displayed both shorter active zones and reduced number of predocked vesicles[57]. Overall, there was a 30–40% decrease in the number of inhibitory active zones per unit length of pyramidal neuron. Such a dramatic decrease would be expected to have severe functional consequences and indeed, the tenascin-R KO mice showed a two-fold decrease in $GABA_A$ receptor-mediated unitary evoked inhibitory postsynaptic current (IPSC) amplitudes and increase in the number of release failures. These effects of tenascin-R deficiency on evoked release occurred with no changes in the size of spontaneous miniature IPSCs, but with changes in their frequency, indicating that the effect is likely to be presynaptic[58].

5.3. Tenascin-R and $GABA_B$ Receptors

The mechanism underlying synaptogenic activity of tenascin-R possibly involves a carbohydrate HNK-1 (originally found on human natural killer cells, hence the name) carried by tenascin-R. Antibodies to this carbohydrate inhibited perisomatic IPSCs, although not dendritic IPSCs or EPSCs[57]. The effects of HNK-1 antibodies were blocked by antagonists to $GABA_B$ receptors and postsynaptic Kir channels coupled to these receptors. Moreover, HNK-1 was found to bind and inhibit recombinant $GABA_B$ receptors[58]. These data led to a model in which tenascin-R via HNK-1 inhibits postsynaptic $GABA_B$ receptor activation under normal conditions. In the tenascin-R KO animals, there is excessive activation of $GABA_B$ receptors, resulting in a postsynaptic activation of Kir channels and the accumulation of K^+ in the extracellular space. This in turn may lead to a chronic depolarization of presynaptic terminals, an increase in spontaneous transmitter release, and a reduction in release probability and IPSC amplitude[58]. An increase in postsynaptic $GABA_B$ receptor-mediated currents in the tenascin-R KO animals supports this model[56]. Further pharmacological analysis or tenascin-R KO may help to verify whether tenascin-R regulates the number of inhibitory synapses via activation of postsynaptic $GABA_B$ receptors.

6. AGRIN

6.1. Structure and Binding Partners of Agrin

The best-studied synaptic organizing ECM molecule is agrin, which is required for formation of the vertebrate skeletal neuromuscular junction (see Chapter 1). Agrin is a large (200 kDa), complex protein that may bear two heparan sulfate chains and therefore is considered to be a heparan sulfate proteoglycan. Agrin is expressed as a secreted protein with the longer N-terminal (LN) or as a transmembrane protein with the shorter N-terminal (SN) domain. Following the SN or LN domains is a set of eight follistatin repeats related to those that bind growth factors and inhibit proteases. The central region of the protein contains two repeats homologous to domain III of laminin chains, a ninth follistatin repeat, two serine/threonine-rich segments. The C-terminal part of agrin, which is responsible for the molecule's known signaling functions, contains four EGF repeats and three so-called G domains homologous to those found in laminin α chains, neurexins, and slits. This C-terminal portion also contains three sites of alternative splicing, called in mammals as x, y, and z. Agrin activates or binds to several membrane- or matrix-associated proteins, including muscle-specific receptor tyrosine kinase, MuSK; laminin γ1 chain; β1 integrins; dystrogycan; NCAM; heparan sulfates; and thrombospondins.

6.2. Agrin's Role in Synaptogenesis

The nerve-derived alternatively spliced z+ isoform of agrin proved to be necessary for postsynaptic differentiation of neuromuscular junction[59]. Synaptogenic function of agrin is mediated by its activation of MuSK. A crucial effector downstream of tyrosine phosphorylation activity mediated by MuSK is the cytoplasmic scaffolding protein rapsyn that induces clustering of acetylcholine receptors, thus providing one prototypic mechanism of ECM action on postsynaptic differentiation (see Chapter 1).

Recent findings indicate that agrin is important for formation of synapses between cholinergic preganglionic axons and sympathetic neurons in the superior cervical ganglion[60] and for formation of splanchnic nerve–chromaffin cell cholinergic synapses in rat adrenal acute slices[61]. In the latter, agrin decreases gap junction-mediated electrical coupling that precedes an increase in nicotinic synaptic transmission. This developmental switch from predominantly electrical to chemical communication is fully operational within 1 hour and depends on the activation of Src family-related tyrosine kinases.

Also in hippocampal cultures, the density of presynaptic boutons and vesicular turnover was reduced when agrin expression or function was suppressed by antisense oligonucleotides and specific antibodies[62,63]. However, synaptogenesis occurred normally in primary hippocampal and cortical neurons derived from agrin-deficient mice[64,65], suggesting the possibility of functional redundancy of agrin and activation of compensatory mechanisms during development in agrin-deficient cultures. Application of recombinant agrin to cultured cortical neurons induces multiple events that involve tyrosine kinase activity and result in modulation of intracellular Ca^{2+} levels and activity of MAPK and CaMKII, phosphorylation of CREB and induction of expression of the immediate early gene

c-fos[66–68]. Conversely, agrin mutants show reduced c-fos expression and resistance to excitotoxicity and seizures. In summary, in several types of synaptic connections, particularly in cholinergic synapses, agrin stimulates activities of tyrosine kinases that affect important aspects of synaptic organization and signaling.

7. LAMININS

7.1. The Laminin Family

Laminins are extracellular glycoproteins that form heterotrimers of various combinations of α, β, and γ subunits that in vertebrates combine to form at least 15 distinct laminins (see ref. 69 and Chapter 1). These heterotrimers assemble into cross-shaped structures consisting of a long α and short β and γ chains, making up each arm of the cross[70–72]. Generally the laminins have conserved domains with either matrix assembly or cell-binding activities. The short arms are often involved in cell matrix assembly and binding while the long α chain can bind to cell surface receptors by either the N- or C-terminal end of the cross. The long α chain may also bind and assemble matrix through an N-terminal globular domain that is present in many, but not all, members of the α chain family. Laminins play multiple roles in vertebrate development, being expressed at very early time points in the embryo. They mediate potent effects on neurite outgrowth, are required for the overall organization of the cortical layers, and play a role in many stages of the brain formation and development[69,73,74]. Because of these early and strong effects of laminins, it had been difficult to tease out their role in CNS synaptogenesis.

7.2. Synaptogenic Activity of Laminins

Like agrin, laminins are known to play an essential role in proper synaptogenesis at the neuromuscular junction[75–78]. There, β2 chain-containing laminins bind directly to the presynaptic P/Q- and N-type calcium channels and induce their clustering, which in turn recruit other presynaptic components. Surprisingly, recruitment occurs independent of Ca^{2+} influx via the channels[75]. Perturbation of laminin-channel interaction *in vivo* results in disassembly of neurotransmitter release sites, active zones, resembling defects previously observed in an autoimmune neuromuscular disorder, Lambert–Eaton myasthenic syndrome. These abnormalities correlate with physiological defects in quantal release and use-dependent modulation of synaptic transmission at the NMJ[79]. Earlier *in vitro* study of ciliary ganglion neurons also showed induction of presynaptic structures by recombinant β2 fragments in the absence of a postsynaptic cell[80]. Thus, it appears that the β2 laminin is capable of initiating a complex set of changes in axons that leads to presynaptic differentiation. Since the N- and P/Q-type calcium channels, to which β2 laminins bind, are widely expressed in CNS, laminins are good candidates to initiate assembly of presynaptic zones in central synapses.

In mice lacking another laminin, α4, active zones and junctional folds of the NMJ form in normal numbers, but not precisely apposed to each other[77], suggesting that laminins may be important players in alignment of pre- and postsynaptic machineries. Abnormalities in rod photoreceptor synapses in laminin

β2 chain-deficient mice[81] also suggest that laminins are important for alignment of pre- and postsynaptic sites. In the normal retina, rod photoreceptors make synapses, called triads, in which three postsynaptic elements invaginate into the base of the photoreceptor such that two horizontal cell dendrites lie laterally, and one bipolar cell dendrite lies centrally. However, in the β2 chain-deficient animals, another type of synaptic configurations, dyads, with only one or two horizontal cell processes apposed to the presynaptic specialization (ribbon), are most common. Also unusually common are floating synapses, wherein a fully formed ribbon, is seen without any postsynaptic element apposed.

8. INTEGRIN'S ROLE IN FORMATION AND MATURATION OF SYNAPSES

Further evidence for the role of laminins in the CNS comes from studies of one of the main laminin receptor classes, the integrins. While there are various laminin receptors including dystroglycan, lectins, and proteoglycans, integrins are the most prevalent and well-studied receptors. As mentioned in Section 3.2, integrins are a family of α and β subunit heterodimeric cell surface receptors expressed throughout the nervous system[74] that mediate a variety of neuronal developmental and functional processes.

Integrins α8, αvβ8, and α3 have all been directly localized to synaptic sites in CNS[82,83]. Since both RGD peptides that block interaction between integrins and their ligands and antibodies to β1 integrins interfered with formation of synapses in organotypic slice cultures, these integrins have been suggested to play a role in synaptogenesis[23]. More evidence on synaptogenic activity of integrins came from a recent work of Hama and colleagues [84] that shows changes in the synaptogenic state of a neuron in response to local contact with astrocytes. Astrocyte contact triggers integrin-mediated activation of protein kinase C throughout the neuron (see Chapter 21). This mechanism may provide a global enhancement of synaptogenesis. This study suggests that integrins may not directly mediate synaptogenesis by acting as cell adhesion molecules at the synapse itself, but rather may act as signaling molecules that change a neuron from either a quiescent or outgrowth state to a synaptogenic state. Such a role would be consistent with some of the known functions of ECM molecules as modifiers of cell function and adhesion through alteration of other adhesion proteins and cell signaling pathways. This study and others[85–88] begin to address a critical, yet poorly understood aspect of CNS synaptogenesis. Namely whether the timing of synapse formation is intrinsically determined by properties of interacting neurons or it may also depend on extrinsic factors such as the ECM. These studies strongly suggest that environmental or ECM signals can have important effects on the timing of CNS synaptogenesis.

Interestingly, during later stages of synaptic development, integrins proved to be necessary for maturation of excitatory hippocampal synapses, converting immature hippocampal synaptic contacts, which express the NR2B subunit of NMDA receptors and have a high glutamate release probability, into mature synapses, which lack NR2B and have lower release probability[21]. Furthermore, numerous studies demonstrated the important role of integrins in synaptic plasticity (for review, see ref. 8).

9. REELIN

9.1. Reelin and Its Receptors

Reelin is a secreted ECM glycoprotein that binds transmembrane receptors on neurons and activates tyrosine kinase signaling cascades[89]. Animals with mutations in Reelin (Reeler mice) strikingly display abnormal neuronal migration such that cortical neurons reverse their normal layering pattern[90]. Reelin signals through two main receptors, the very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2). Both of these receptors appear to signal through the disabled (Dab-1) signaling pathway to mediate Reelin's effects on neuronal migration[91]. In addition, there is evidence that Reelin can signal through $\alpha 3\beta 1$ integrin-dependent pathways as well[92].

9.2. Reelin's Role in Synaptogenesis

In addition to an effect on migration, studies have also linked Reelin to synapse formation in the hippocampus. Although hippocampal layering is largely unaffected in the Reeler mouse, defects are seen both in axonal pathfinding and synaptogenesis in the hippocampus[93,94]. Hippocampal entorhinal projections are topographically correct in Reeler mice, but more fibers make aberrant projections, there are fewer branches of entorhinal axons, and there is a nearly 50% reduction in the number of synapses at P2 and a 30% reduction at P12[94]. Also heterozygous Reeler mice exhibit a decrease of dendritic spine density on cortical and CA1 pyramidal neurons of the hippocampus[95]. It is difficult to verify if Reelin is playing a direct role in hippocampal synaptogenesis or if the reduction is due to poorly developed dendritic trees or the reduced branching and misrouting of the entorhinal projections. Multiple mechanisms for Reelin in synaptogenesis are likely because there are fewer synaptic varicosities per length of axon in Reeler mice[94], Reelin is expressed in CNS synapses and affects synaptic properties[96] as well as dendritic development[97].

Another intriguing example of a possible role for Reelin in synaptogenesis comes from studies of the retina. Reelin is expressed by retinal ganglion cells, amacrine cells, and rod bipolar cells in the developing and adult retina[98]. Reelin is also strongly expressed in the synaptic inner plexiform layer of the retina and as this layer matures into its distinct ON/OFF sublaminae (see ref. 99 for review), Reelin becomes localized predominantly to the ON layer[92]. In the Reeler mouse, a subset of Reelin expressing rod bipolar cells terminate in the incorrect layer of the retina and do not synapse in the ON sublamina. Thus, Reelin may play a role in the establishment of appropriate synaptic circuitry in the retina perhaps by modulating the relative adhesiveness of proteins involved in synaptic specificity.

9.3. Reelin and Synaptic Maturation

Several studies suggest that Reelin affects synaptic functions via VLDL and apoER2 receptors which may induce mDab1 tyrosine phosphorylation and activation of nonreceptor tyrosine kinases of the Src family. This idea is supported by recent data on maturation of synapses in hippocampal cultures[96]. In this system, chronic blockade of the function of Reelin with antisense oligonucleotides or the

function-blocking antibody prevented the decrease of NR1/2B-mediated whole-cell currents, which is a hallmark of synaptic maturation. Conversely, exogenously added recombinant Reelin accelerated the maturational changes in NMDA-evoked currents. Importantly, the change in NMDAR subunits was also blocked by chronic treatment with an inhibitor of the Src kinase signaling pathway or an antagonist of the LDL receptors, receptor associated protein[96]. Even stronger evidence supports a link between Reelin, apoER2, tyrosine phosphorylation of NMDA receptors, and long-term potentiation[8]. Thus, signaling via apoER2/VLDL receptors and tyrosine phosphorylation appeared to be critical in mediating effects of Reelin on synaptic functions. Whether the same mechanisms are operant during stabilization of nascent synapses remains to be investigated. The importance of the above-described findings is underscored by reduction of Reelin expression in psychotic patients and similarities in some cellular and synaptic abnormalities observed in these patients and Reeler mice.

10. CONCLUSIONS

The findings discussed in this chapter suggest that ECM molecule may shape synaptogenesis via several mechanisms. On the one hand, they may aggregate and serve as scaffolds for accumulation of presynaptic Ca^{2+} channels, postsynaptic glutamate receptors and growth factors (Figure 11.3).

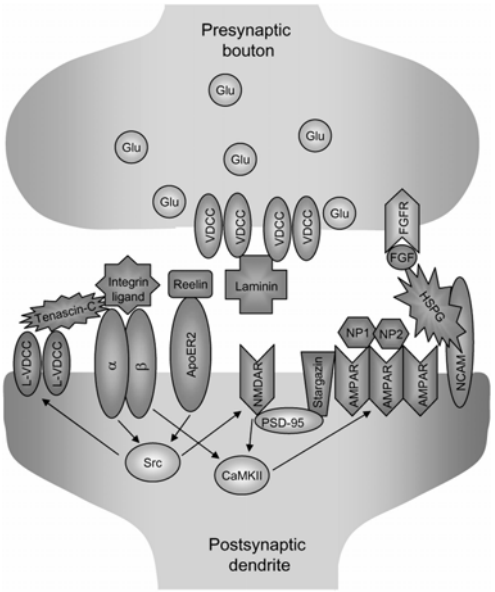

Figure 11.3. Hypothetical Mechanisms by which ECM Molecules Shape Synaptogenesis. Neuronal pentraxins, NP1 and NP2, may form scaffolds in the extracellular space, thus promoting clustering of postsynaptic AMPA subtype of glutamate (Glu) receptors (AMPARs) and associated proteins, such as Stargazin, PSD-95, and NMDA receptors (NMDARs). Scaffold-containing laminins cluster presynaptic voltage-dependent Ca^{2+} channels (VDCC). Similarly, heparan sulfate proteoglycans (HSPGs) in association with NCAM serve to accumulate FGF and amplify signaling via FGF receptors. Reelin and laminin may bind to their cell surface receptors (ApoER2 and integrins) and trigger intracellular cascades resulting in activation of tyrosine kinases of the Src family and tyrosine phosphorylation of effectors, including NMDA receptors (NMDAR).

Data on laminin and neuronal pentraxins spectacularly illustrate this mechanism. Additionally, a recent study on synaptogenic activity of the neural cell adhesion molecule NCAM shows that it is related to formation of a complex between polysialylated NCAM and heparan sulfate proteoglycans and signaling via fibroblast growth factor receptor (see Chapter 6). On the other hand, ECM

molecules may serve as ligands capable of activating intracellular signaling cascades via binding to cell surface receptors. A common feature of these signaling events is tyrosine phosphorylation of effectors involved in synaptic transmission and plasticity.

The ECM molecules and receptors mentioned above are by no means the only ones involved in synapse formation and function. An increasing number of ECM signals are now being implicated in these processes such as aggrecan, neurocan, brevican, versican and HB-GAM (see ref. 100 for review). In addition, ECM components such as F-spondin may modify protease function and lead to altering processing and breakdown of neuronal proteins implicated in synapse formation and function, such as amyloid precursor protein[100,101]. Thus, it is becoming clear that ECM may play a role in synaptogensis by modifying the adhesion of neuronal synaptogenic proteins, diffusional parameters of the extracellular space[102], the response of neurons to growth factors[73], and the synaptogenic state of neurons. How ECM does this and what receptors and signaling pathways are involved are questions still mostly waiting for answers.

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