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Organization of central synapses by adhesion molecules

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Abstract

Synapses are the primary means for transmitting information from one neuron to the next. They are formed during development of the nervous system, and formation of appropriate synapses is crucial for establishment of neuronal circuits that underlie behavior and cognition. Understanding how synapses form and are maintained will allow us to address developmental disorders such as autism, mental retardation and possibly also psychological disorders. A number of biochemical and proteomic studies have revealed a diverse and vast assortment of molecules present at the synapse. It is now important to untangle this large array of proteins and determine how it assembles into a functioning unit. Here we focus on recent reports describing how synaptic cell adhesion molecules interact with and organize the pre- and postsynaptic specializations of both excitatory and inhibitory central synapses.

Keywords

development; postsynaptic density; neuroligin; neurexin; SynCAM; ephrin

INTRODUCTION

As synapses are specialized sites of contact between neurons that facilitate neurotransmission, their organization must be tightly regulated. Cell adhesion molecules (CAMs) may serve to both facilitate the organization and the adhesion of the synapse. Indeed, similar to other adhesion sites such as adherens junctions and tight junctions, synapses contain some of the same CAMs. For example, integrins and cadherins, both key players at tight junctions, are prevalent at synapses and are integral to their correct structure and function (Benson *et al.*, 2000; Chavis & Westbrook, 2001; Togashi *et al.*, 2002; Arikkath & Reichardt, 2008). They help to recruit and organize key components such as synaptic vesicles at the presynaptic terminal and neurotransmitter receptors (NTRs) in the postsynaptic specialization.

However, many additional CAMs have evolved, adding great diversity to the types of adhesion molecules at the synapse; including the neuroligins (Nlgns), the nectin-like synaptic CAMs (SynCAMs), synaptic adhesion-like molecules (SALMs), netrin-G-ligands (NGLs), leucine-rich repeat transmembrane proteins (LRRTMs), ephrin receptors (Eph) and Sidekicks (Table 1; for reviews see Yamagata *et al.*, 2003; Washbourne *et al.*, 2004a; Gerrow & El-Husseini, 2006; Dalva *et al.*, 2007; Brose, 2009). The Nlgns were identified as

trans-synaptic partners of neurexins (Ichtchenko *et al.*, 1995). Prior to this, the neurexins were only known to interact with the black widow spider toxin, α-latrotoxin (Davletov *et al.*, 1995). Subsequent studies demonstrated that adhesion-induced clustering of neurexins in axons leads to synaptic vesicle immobilization (Scheiffele *et al.*, 2000; Dean *et al.*, 2003), while clustering of Nlgns results in recruitment of postsynaptic components (Nam & Chen, 2005; Barrow *et al.*, 2009). Since the neurexin/Nlgn adhesion pair was the first such synaptogenic interaction to be characterized, it served as a template for the subsequent identification of other synaptic cell adhesion molecules (Biederer & Scheiffele, 2007).

The SynCAMs bind heterophilically to other SynCAM family members through their extracellular immunoglobulin (IG) domains (Fogel *et al.*, 2007), although which SynCAM family member is localized to the pre- or postsynaptic side remains vague. Comparison of the synaptogenic activity of SynCAMs with Nlgns revealed that both are able to induce equivalent presynaptic structures, however, only Nlgn1 induces postsynaptic structures as detected by immunolabeling, whereas only SynCAM1 induces increased synaptic activity as measured by electrophysiological techniques (Sara *et al.*, 2005). SALMs were identified as molecules that could interact with the postsynaptic density protein PSD95 and their overexpression results in the formation of additional excitatory synapses (Ko *et al.*, 2006a). NGLs were identified using a very similar approach to SALMs (Kim *et al.*, 2006). Both SALMs and NGLs are heterophilic molecules, binding a variety of presynaptically localized membrane proteins, notably netrins, LAR and reticulon3 (Table 1) (Woo *et al.*, 2009b). An expression screen led to the identification of the LRRTM proteins as inducers of synaptic vesicle recruitment (Linhoff *et al.*, 2009), and recent studies have identified these as being postsynaptic ligands to the neurexins (de Wit *et al.*, 2009; Ko *et al.*, 2009). The binding of ephrins to their receptors, the EphAs and EphBs, had long been known to direct axon pathfinding (Flanagan & Vanderhaeghen, 1998). However, the identification of the recruitment of NMDA receptors to synapses through a direct interaction with EphBs (Dalva *et al.*, 2000) and the demonstration of trans-synaptic induction of presynaptic specializations unveiled the synaptogenic potential of the ephrin/EphB pair (Kayser *et al.*, 2006). Finally, the sidekicks were identified as homophilic adhesion molecules that mediate lamina-specific targeting of synaptic connectivity in the retina (Yamagata *et al.*, 2002).

Thus, over the past decade, the number of synaptic CAMs identified has steadily increased (with more potentially waiting to be uncovered) and they have all been subjected to increased scrutiny by means of biochemical, genetic and cell biological analyses (Brose, 2009). However, the major task ahead is now to elucidate the mechanisms by which these molecules may instruct the formation and maintenance of synapses. In this review, we focus our attention on recent studies which commence to shed light on the molecular interactions of CAMs that shape the developing synapse and determine the molecular organization of the mature synaptic contact. First, we will consider what is known about molecular interactions in the presynaptic terminal and then focus on the postsynaptic specialization.

HOOKING UP WITH SYNAPTIC VESICLES

The majority of synaptic CAMs were identified based on their ability to cluster synaptic vesicles in axons at sites of contact (Scheiffele *et al.*, 2000; Biederer *et al.*, 2002; Linhoff *et*

al., 2009). Such an assay is performed by immunolabeling neurons cocultured with non-neuronal cells, such as HEK293 cells, expressing potential synaptic CAMs (Scheiffele *et al.*, 2000). Quantification of the intensity or area of synaptic markers on the transfected cells versus non-transfected cells gives a measure of how well the CAM can cause immobilization of synaptic vesicles (Biederer & Scheiffele, 2007). However, while the majority of the synaptic CAMs are capable of recruiting synaptic vesicles (Dalva *et al.*, 2007), it remains unclear exactly how this recruitment occurs. A few studies which show interactions of synaptic CAMs with presynaptic components converge on the Veli/CASK/Mint1 complex. This extremely stable tripartite complex is highly enriched in synaptic membrane fractions (Butz *et al.*, 1998). Veli, CASK and Mint1 bind to each other through their N-terminal domains, leaving their PDZ domains free to interact with other proteins, such as Munc18-1, a key regulator of synaptic vesicle exocytosis (Butz *et al.*, 1998). Although Mint1 and Munc18-1 localize to active zones and to a lesser extent synaptic vesicles in the molecular layer of the cerebellum (Okamoto *et al.*, 2000), a clear function for the tripartite complex in presynaptic assembly has yet to be demonstrated. Nevertheless, β-catenin, the principle interacting protein of cadherins (Yap *et al.*, 2007; Arikkath & Reichardt, 2008), can bind to Veli through a PDZ interaction motif and this interaction is important for synaptic localization of the reserve pool of synaptic vesicles (Bamji *et al.*, 2003).

Neurexins, the presynaptic ligands of Nlgns, can bind to CASK also through a PDZ binding motif (Hata *et al.*, 1996). This interaction links the neurexin-Nlgn synaptic adhesion complex to synaptic vesicle exocytosis through the tripartite complex (Butz *et al.*, 1998). More recent studies have gone on to show that Mint1 can be displaced by caskin1, and that the two resulting alternative tripartite complexes (i.e. Veli/CASK/Mint1 and Veli/CASK/caskin1) are equivalent in their relative amounts in brain homogenates (Tabuchi *et al.*, 2002). It remains unclear, though, whether these alternative tripartite complex have different functional significances for organization of the presynaptic terminal. In addition, it is important to note that CASK can also potentially interact with rabphilin3a, a protein important for regulating exocytosis of synaptic vesicles, through the guanylate kinase-like domain of CASK (Zhang *et al.*, 2001). Many more questions remain regarding presynaptic assembly. How does the interaction with the Veli/CASK/Mint1 or Veli/CASK/caskin1 complex attract and stabilize presynaptic components at a nascent synaptic site? This question is particularly relevant now, since the Veli/CASK/Mint1 complex has been implicated in postsynaptic assembly, as well as at the presynaptic terminal. The Veli/CASK/Mint1 complex forms the link between NMDA-type ionotropic glutamate receptors and the motor protein KIF17 (Setou *et al.*, 2000). Furthermore, how is the recruitment and maintenance of synaptic vesicles, their fusion machinery and other active zone components regulated?

One indication of how the active zone cytomatrix may be recruited to synapses comes from a recent study of the presynaptic effects of ephrin-B1 and B2 (McClelland *et al.*, 2009). These transmembrane receptors of the EphBs bind to syntenin-1 through a PDZ domain interaction and cause syntenin-1 to accumulate at synaptic contacts (McClelland *et al.*, 2009). Syntenin-1 has been linked to presynaptic maturation via ERC2/CAST1 (Ko *et al.*,

2006b). ERC2/CAST1 interacts with a number of very large proteins, including Piccolo and Bassoon, which form part of the active zone cytomatrix (Jin & Garner, 2008). This suggests that each presynaptic CAM family may contribute to the recruitment of different integral components of the presynaptic terminal.

THE LINK TO NEUROTRANSMITTER RECEPTORS

In contrast to this relative paucity in the understanding of interactions between CAMs and presynaptic components, many more studies have revealed a wealth of potential mechanisms through which CAMs may organize the postsynaptic specialization. There are several synaptic characteristics for which CAMs may be important determinants, ranging from the size of the synaptic cleft to the cytoskeletal structure of the spines of excitatory synapses. However, the key functional property of the postsynaptic specialization is the presence of NTRs. Thus, a major synaptic CAM function, that has a direct impact on neuronal physiology, is the ability to direct or maintain the aggregation of NTRs or of NTR-associated proteins at the synapse. There are at least three ways in which CAMs could perform this organizational function (Figure 1). They can (a) interact directly with NTRs, (b) interact with scaffolding molecules which in turn bind NTRs or (c) initiate signaling cascades which lead to the recruitment or maintenance of NTRs at synapses. Recent studies demonstrate that synaptic CAMs have co-opted all of these possibilities in organizing the postsynaptic specialization.

Direct interaction

To date, the EphB receptors are the only synaptic CAMs for which a direct interaction with NTRs has been demonstrated. These receptor tyrosine kinases (RTK) bind to NMDA-type glutamate receptor subunit 1 (NMDAR1; Dalva *et al.*, 2000). Interestingly, this interaction is entirely dependent on EphB2 binding to its trans-synaptic ligand, ephrinB2 (Figure 1a). The interaction is mediated by the extracellular domain of EphB2 binding to the extracellular N-terminus of NMDAR1 (Dalva *et al.*, 2000). As expected, this direct interaction stabilizes NMDARs at synaptic sites; overexpression of EphB2 in cultured cortical neurons or incubation with aggregates of soluble ephrinB2 results in a higher density of NMDAR1 immunoreactive clusters along neuronal dendrites (Dalva *et al.*, 2000). While binding of ephrinB2 to EphB2 results in activation of the RTK, its enzymatic activity is not necessary for the interaction with the NMDA receptor.

It is entirely possible that the EphBs are not the only synaptic CAMs to bind NTRs through their extracellular domains; however, this possibility has remained underexplored. The SynCAMs possess 3 IG domains and a highly variably spliced domain (Biederer, 2006). The first two IG domains are necessary for the trans-synaptic interaction (Fogel *et al.*, 2007), leaving an orphan IG and variable domain in the extracellular space. It will be interesting to determine whether these regions can potentially interact with other synaptic proteins such as NTRs. Similarly, Nlgns possess a stem region between the acetylcholinesterase domain and the transmembrane domain for which a function has not yet been determined. Studies in cultured neurons suggest that known intracellular protein-protein interactions do not explain all possible activities for the recruitment of NMDA receptors to synapses. Deletion of the PDZ motif of Nlgn1 which can interact with a multitude of scaffolding proteins (see below)

does not completely abrogate its ability to induce the clustering of NMDA receptors (Chih *et al.*, 2005) or trafficking with NMDA receptors (Barrow *et al.*, 2009). This may suggest a potential interaction with this receptor through the extracellular domain of Nlgn1. Also, the extracellular domains of other synaptic CAMs, such as the netrin-G-ligands (NGLs) or the leucine-rich repeat transmembrane proteins (LRRTMs), contain multiple protein interaction domains for which ligands have yet to be determined, and which could, therefore, potentially interact with key synaptic proteins such as NTRs.

Scaffolding protein interactions

Interaction via PDZ motifs—An intracellular interaction domain that has long been recognized as a major player in organizing the postsynaptic zone is the PDZ domain (Garner *et al.*, 2000). This domain, which was named for the first three proteins identified to contain the domain (PSD95, discs large and zona occludens-1), is comprised of around 90 amino acids in two α helices and six β sheets (Garner *et al.*, 2000). A great number of intracellular proteins that have been localized specifically to the postsynaptic compartment contain either single or multiple copies (up to 7) of the PDZ domain (Garner *et al.*, 2000). As many of these proteins contain multiple protein-protein interaction domains, they have become collectively termed scaffolding proteins (Sheng & Kim, 2000). Furthermore, a subset of these intracellular scaffolding proteins, which present an inactive guanylate kinase domain, are classed together as membrane associated guanylate kinase proteins (MAGUKs; Funke *et al.*, 2005).

PDZ domains most commonly bind to short motifs located at the C-termini of other proteins. These motifs can be loosely divided into two categories. Type I motifs have a small hydrophobic residue at the very C-terminus (0 position) and either a serine or threonine (or possibly cysteine) at the −2 position, whereas type II motifs have hydrophobic residues at both −2 and 0 positions (Kang *et al.*, 2003). The majority of synaptic CAMs studied to date present PDZ motifs at their C-termini (Table 2). Furthermore, a large number of NTRs present PDZ binding motifs in their intracellular tails. Thus, one can envisage trimer complexes between a CAM and an NTR linked by a multiple PDZ domain containing scaffolding molecule (Figure 1b). However, a number of studies make it clear that interactions are overlapping and plentiful (Table 2; Torres *et al.*, 1998; Garner *et al.*, 2000; Meyer *et al.*, 2004). Consequentially, one has to imagine a complex web of interactions incorporating different CAMs, scaffold proteins and NTRs at a single synapse (Garner *et al.*, 2000).

A few studies have employed a yeast two hybrid (Y2H) approach to uncover potential PDZ interactions of synaptic CAMs (Torres *et al.*, 1998; Meyer *et al.*, 2004). These studies have permitted the comparison of PDZ interactions across multiple CAMs (Nlgn1 and 2, SynCAM1, ephrinB2, Sidekick2; Meyer *et al.*, 2004). Indeed, they have provided a wealth of potential interactions (Table 2), which must now be validated. It is possible that interactions within the nuclei of yeast cells do not recapitulate the interactions seen at the plasma membrane in mammalian cells. For example, while GRIP was identified as an interacting protein of the PDZ motif of SynCAM1 (called IGSF4 in that study) through a

Y2H screen (Meyer *et al.*, 2004), this interaction has not been reproduced in a mammalian cell interaction assay (Hoy *et al.*, 2009).

The most extensively studied intracellular interactions belong to the Nlgns (Irie *et al.*, 1997; Meyer *et al.*, 2004). Here, also, the validation of these interactions lags considerably behind the biochemical interaction possibilities, with only the interaction of the third PDZ domain of PSD95 having received significant attention in a neuronal context (Prange *et al.*, 2004; Chih *et al.*, 2005; Nam & Chen, 2005; Heine *et al.*, 2008; Barrow *et al.*, 2009). Clustering of Nlgn1 at the surface of neurons either by overexpression (Chih *et al.*, 2005), by presentation of neurexin from a non-neuronal cell (Nam & Chen, 2005), by providing neurexin-coated beads (Heine *et al.*, 2008) or by incubation with soluble neurexin multimers (Barrow *et al.*, 2009) results in the aggregation of PSD95. This indicates that trans-synaptic binding of the neurexin/Nlgn complex is instructional for the recruitment of the postsynaptic protein PSD95 with a rapid time course (Heine *et al.*, 2008; Barrow *et al.*, 2009). The time delay between clustering of Nlgn1 and the recruitment of PSD95 (on the order of 1 hour) indicate that PSD95 is not pre-bound to the Nlgn1 intracellular tail (Barrow *et al.*, 2009). Interestingly, palmitoylation is necessary for PSD95 aggregation at synaptic sites (El-Husseini *et al.*, 2002) and at Nlgn1 clusters (Barrow *et al.*, 2009), and this slow enzymatic step may account for the delay in recruitment. However, it is important to note that Nlgn1 and PSD95 may exist in pre-existing clusters at non-synaptic dendritic locations (Gerrow *et al.*, 2006). These observations suggest that bringing Nlgn1 into clusters at the neuronal cell surface may trigger a signal that results in the palmitoylation of PSD95, allowing PSD95 to interact with the plasma membrane and increasing the likelihood of interacting with the PDZ motif of Nlgn1 (Washbourne, 2004; Huang & El-Husseini, 2005). The exact order of events is still not clear and resolving this will be highly informative of how recruitment of scaffold proteins to synapses occurs.

Clustering of Nlgn1 results in the accumulation of NMDA-type and AMPA-type glutamate receptors, too (Nam & Chen, 2005; Heine *et al.*, 2008). Given that PSD95 binds the NMDA receptor through its first and second PDZ domains (Kornau *et al.*, 1995), one could imagine a hypothetical trimeric complex consisting of Nlgn1, PSD95 and the NMDA receptor (Figure 1b). However, the NMDA receptor remains associated with Nlgn1 during trafficking and aggregates at the synapse even when the C-terminal PDZ motif of Nlgn1 has been deleted (Chih *et al.*, 2005; Barrow *et al.*, 2009). This redundancy may be explained by an interaction between synaptic scaffolding molecule (S-SCAM, or MAGI2), which can bind Nlgn1 via a WW domain (Iida *et al.*, 2004), and NMDA receptors (Iida *et al.*, 2007). Alternatively, there is the possibility of a yet unidentified interaction between Nlgn1 and NMDARs. Additional experiments monitoring aggregation of PSD95 to neurexin-coated beads coupled with electrophysiological recordings suggest that neurexin-Nlgn1 adhesion results in the recruitment of AMPA-type glutamate receptors (Heine *et al.*, 2008). The specific interactions downstream of Nlgn1 that might be mediating the recruitment of AMPA receptors remain unclear, but may involve either PSD95 (El-Husseini *et al.*, 2000) or an association with transmembrane AMPA receptor regulatory proteins (TARPs) through S-SCAM (Deng *et al.*, 2006).

Induction of PSD95 aggregation by clustering synaptic CAMs appears to be a common theme (Table 2) as NGLs, SALMs and LRRTMs all interact with PSD95 and cause it to form puncta at synapses when the cognate trans-synaptic ligand is applied to neurons in culture (Kim *et al.*, 2006; Ko *et al.*, 2006a; Han & Kim, 2008; Linhoff *et al.*, 2009). It is unclear whether PSD95 is the primary PDZ domain protein that these synaptic CAMs interact with or whether it has been chosen due to its status as postsynaptic marker *extraordinaire* for the glutamatergic synapse. If PSD95 is the major interacting protein for at least 4 different families of synaptic CAMs, PSD95 would be a linchpin for the formation and maintenance of the postsynaptic density (Han & Kim, 2008). Overexpression of PSD95 drives AMPA receptors to the synapse (El-Husseini *et al.*, 2000; Beique *et al.*, 2006), and ablation of the PSD95 gene in mice results in enhanced NMDA receptor-dependent LTP (Migaud *et al.*, 1998). While these data suggest an important role for PSD95 in regulating synaptic strength, they do not point to PSD95 being the central organizer of glutamatergic synapses. Given the large number of MAGUKs with a very similar domain structure to PSD95 (such as PSD93, SAP97 etc), it is possible that PSD95 is only one of a host of scaffolding proteins that all act together as central organizers of glutamatergic synapses. Future studies will have to examine more closely whether the identity of the PSD95-like MAGUK that is primarily recruited by a particular synaptic CAM gives a synapse a particular ‘flavor’, i.e. an underlying property such as synaptic strength or signaling capabilities.

It is important to consider the consequence of the binding of PDZ domain-containing proteins for CAMs. As mentioned above, it is the binding of the trans-synaptic ligands of Nlgn1, LRRTM1 and 2, SALM2 and NGL1 which drives the formation of PSD95 clusters. However, disruption of the PDZ motif in Nlgn1, SALMs, NGLs and LRRTMs does not affect the synaptic localization of the CAMs themselves (Rosales *et al.*, 2005; Kim *et al.*, 2006; Ko *et al.*, 2006a; Linhoff *et al.*, 2009). In contrast, removal of additional sequences in the C-terminal tails of the Nlgn1 and LRRTM1/2 does disrupt their localization to synapses (Rosales *et al.*, 2005; Linhoff *et al.*, 2009). Thus, interactions via alternative protein motifs, for example with the WW domain of S-SCAM in the case of Nlgn1 (Iida *et al.*, 2004), are important for directing the localization of CAMs to synapses. Also, PDZ interactions with MAGUK proteins other than PSD95, such as SAP102 or SAP97, mediate cotransport of glutamate receptors with Nlgn1 (Sans *et al.*, 2003; Washbourne *et al.*, 2004b; Barrow *et al.*, 2009). Thus, deletion of the PDZ motif in Nlgn1 does not disrupt the localization of Nlgn1 (Rosales *et al.*, 2005), but does significantly change co-transport with glutamate receptors (Barrow *et al.*, 2009).

Interaction via non-PDZ motifs—While PDZ domain interactions are numerous within the postsynaptic specialization (Garner *et al.*, 2000), a number of protein-protein interactions through other domains have recently been uncovered at synaptic CAMs. Importantly, an interaction between Nlgn2 and gephyrin has been characterized at inhibitory synapses (Poulopoulos *et al.*, 2009). Nlgn2 is one of the few synaptic CAMs that localizes almost exclusively to GABAergic synapses (Graf *et al.*, 2004; Varoqueaux *et al.*, 2004; Chih *et al.*, 2005; Levinson *et al.*, 2005). Gephyrin, a key scaffolding molecule of glycinergic and GABAergic synapses (Kneussel & Betz, 2000), has the potential to bind to a 15 amino acid

stretch in the intracellular C-tail present in all Nlgns via the E-domain of gephyrin (Poulopoulos *et al.*, 2009). However, it was recently uncovered that a specific interaction between collybistin and Nlgn2 activates the protein collybistin (Poulopoulos *et al.*, 2009). This activation involves collybistin's src homology domain 3 (SH3), and enables gephyrin to be recruited to the plasma membrane (Harvey *et al.*, 2004). The interaction with collybistin generates the specificity necessary for Nlgn2 to be the only Nlgn with the ability to interact with inhibitory NTRs.

Recently, another interaction at inhibitory synapses was discovered. It had previously been shown that S-SCAM (or MAGI2) has the potential to interact with Nlgn1 (Iida *et al.*, 2004) and with the NMDA receptor (Iida *et al.*, 2007), an interaction that may account for trafficking of NMDA receptors with Nlgn1 in dendrites (Barrow *et al.*, 2009). However, one third of S-SCAM clusters in cultured hippocampal neurons are located at inhibitory synapses (Sumita *et al.*, 2007). This localization is mediated by the binding of S-SCAM to β-dystroglycan, a glycoprotein located specifically at inhibitory synapses (Levi *et al.*, 2002). This interaction is mediated by the three WW domains of S-SCAM. These domains are triple stranded beta sheets which bind to proline-rich motifs (Ilsley *et al.*, 2002) and also mediate the interaction with Nlgn2 (Sumita *et al.*, 2007). Thus, a versatile scaffold molecule links Nlgn1 to glutamate receptors at excitatory synapses and also links Nlgn2 to the dystroglycan complex at inhibitory synapses.

The SynCAMs, which are encoded by the CADM gene family (Biederer, 2006; Pietri *et al.*, 2008), belong to the nectin-like molecules (Takai *et al.*, 2008). These homophilic CAMs mediate cell adhesion events in many non-neuronal tissues (Fujita *et al.*, 2006; Takai *et al.*, 2008), but were also implicated in mediating synaptogenesis (Biederer *et al.*, 2002). While it was clear that SynCAM1 could drive the differentiation of the presynaptic terminal by recruiting synaptic vesicles (Biederer *et al.*, 2002) by an as yet unknown mechanism, it was only recently that a postsynaptic interaction was uncovered. Like other synaptic CAMs, SynCAMs possess a PDZ motif, which can potentially interact with CASK and syntenin (Table 2). However, it is the binding of members of the 4.1 family of proteins (4.1N and 4.1B) via their FERM domains to a juxtamembrane motif of SynCAM1 that drives the recruitment of glutamate receptors (Hoy *et al.*, 2009). 4.1 proteins are known for their functions at cellular adhesion sites where they can recruit the actin cytoskeleton through a spectrin-actin binding domain and bind to transmembrane proteins via the FERM domain (named for its presence in 4.1 proteins, ezrin, radixin and moesin; Hoover & Bryant, 2000). Protein 4.1N interacts directly with AMPA receptor subunit GluR1 through the C-terminal domain (Shen *et al.*, 2000), while 4.1B can specifically recruit NMDA receptors to SynCAM1 adhesions through an unknown interaction (Hoy *et al.*, 2009). FERM binding motifs are present in other synaptic CAMs, notably in the intracellular tail of neurexins, where it was proposed that binding of 4.1 proteins would mediate an interaction with the actin cytoskeleton (Biederer & Sudhof, 2001). The binding of FERM domain-containing proteins to cell adhesion molecules is evolutionarily conserved: coracle and yurt, which are related to the 4.1 proteins, bind to neurexin IV in *Drosophila melanogaster* and act to establish epithelial polarity (Laprise *et al.*, 2009). Thus, 4.1 protein interactions with

synaptic CAMs have the potential to organize the synapse both through direct interactions with NTRs and also by recruiting the cytoskeleton.

Cadherins were thought, for a long time, to only contribute to the postsynaptic specialization in a structural capacity, by virtue of their localization at the periphery of postsynaptic densities (Fannon & Colman, 1996; Elste & Benson, 2006). However, a chain of scaffolding molecule interactions linking the cadherins to AMPA-type glutamate receptors was recently discovered. Cadherins bind δ-catenin, also known as neural plakophilin-related arm protein (NPRAP). This molecule, in turn, interacts with AMPA-receptor binding protein (ABP) and GRIP, multi-PDZ domain proteins that bind AMPA receptor subunits (Silverman *et al.*, 2007). Thus, negatively affecting the link from cadherin to ABP/GRIP reduces the number of GluR2 subunits at synapses.

Signaling Interactions

The synaptic CAMs for which the transduction of cell-cell contact results in an enzymatic signaling cascade is most apparent are the EphBs. They possess an intracellular tyrosine kinase domain, which is activated on binding the trans-synaptic ligands, ephrinBs (Kullander & Klein, 2002). Furthermore, they can activate non-receptor tyrosine kinases and GTPases through guanine nucleotide exchange factors (GEFs; Kullander & Klein, 2002). EphrinB binding by EphB2 results in the phosphorylation of Rac1-GEF Tiam1 enabling spine formation (Tolias *et al.*, 2007). Furthermore, activation of the Rho-GEF kalirin induces the localization of p21 activated kinase (PAK) to synapses, resulting in spine morphogenesis (Penzes *et al.*, 2003). Recent work suggests that it is signaling by EphBs that maintains the mobility of dendritic filopodia and that, upon trans-synaptic contact with ephrinBs, the activity of PAK causes filopodia to then transition to stable spine structures (Kayser *et al.*, 2008). Indeed, researchers were able to rescue both decreased filopodial motility and synaptogenesis in EphB mutant cortical slice cultures by introducing either wildtype EphB2 or mutant EphB2 presenting only the extracellular domain together with constitutively active PAK (Kayser *et al.*, 2008). In addition, EphB2 can recruit focal adhesion kinase (fak) to modulate spine morphogenesis (Moeller *et al.*, 2006). Thus, the EphBs are a prototypical synaptic CAM, in that they are able to drive differentiation of the postsynaptic specialization of glutamatergic synapses through all three molecular mechanisms (Figure 1): (a) direct interaction with NMDA receptors, (b) indirect interaction with AMPA receptors through GRIP (Torres *et al.*, 1998), Table 2) and (c) activation of enzymatic signaling cascades through the GEFs kalirin and Tiam1. Importantly, EphBs also provide a molecular link between the necessity for both filopodial motility and trans-synaptic adhesion during synaptogenesis.

However, EphBs are not the only synaptic CAMs to signal through signaling cascades. Recently, cadherins were also shown to activate PAK through kalirin-7, resulting in spine growth and increased AMPA receptor content (Xie *et al.*, 2008). Kalirin-7 is recruited to cadherins through the scaffolding protein afadin (or AF-6). This scaffolding protein binds to kalirin-7 through its single PDZ domain (Xie *et al.*, 2008). It remains unclear, though, how afadin then binds to cadherins, as it had previously been suggested to bind to cadherins

through this same PDZ domain (Mandai *et al.*, 1997). It is possible that afadin forms a dimer to mediate this interaction.

The Nlgns have also been implicated in triggering signaling cascades. Epac2 (exchange factor directly activated by cAMP), a GEF, was found to modulate spine motility and to induce the removal of AMPA receptor subunits from the synapse (Woolfrey *et al.*, 2009). This is triggered by an association with Nlgn3 and the downstream effects are mediated by the stimulation of Rap activity (Woolfrey *et al.*, 2009). Interestingly, the spine shrinkage was dependent on dopamine receptor D1 activity. Thus, Nlgns may now be linked to cAMP-mediated and dopamine receptor-mediated long-term depression (LTD) and provide further insights into the etiology of autism spectrum disorders, as mutations in the Nlgn3 and Epac2 genes are associated with autism (Bacchelli *et al.*, 2003; Jamain *et al.*, 2003).

Additionally, Nlgns associate with the protein tyrosine phosphatase receptor T (PTPRT; Lim *et al.*, 2009). Overexpression of this transmembrane protein phosphatase in cultured hippocampal neurons increased both excitatory and inhibitory synapse formation. This activity was dependent on an interaction between the extracellular domain of PTPRT with the extracellular domain of Nlgns (Lim *et al.*, 2009). Furthermore, the activity of PTPRT was modulated by phosphorylation of the intracellular catalytic domain by the kinase Fyn. It remains unclear what the downstream substrates of the phosphatase activity of PTPRT are, however this study highlights how an extracellular interaction (Figure 1a) brings about a signaling cascade (Figure 1c) that can drive synaptogenesis. Further studies may uncover how this phosphatase may regulate both excitatory and inhibitory synapse formation.

Interestingly, the observation that collybistin interacts with Nlgn2 to specifically recruit gephyrin and therefore GABA A and glycine receptors to inhibitory synapses may hint at an additional signaling cascade through Nlgns, as collybistin is also a GEF protein (also known as ARHGEF9). In fact, it was first hypothesized that the GEF activity was necessary for recruitment of gephyrin and glycine receptors into aggregates (Kins *et al.*, 2000). It is now understood that the interaction between collybistin and gephyrin is direct and does not require the guanine nucleotide exchange activity (Grosskreutz *et al.*, 2001). It will be interesting to test whether the GEF activity is important for some other aspect of the development of inhibitory synapses.

SYNERGY OR REDUNDANCY?

Thus, CAMs are intricately entwined into the complex macromolecular structure that lies on the postsynaptic side of every synapse. They are connected to the NTRs, either directly or indirectly via scaffolding molecules or indirectly via signaling cascades (Figure 1). CAMs are presumably even connected to each other through these various modes of interaction. The question then arises as to whether they work in concert to bring about the formation and the maintenance of the synapse. Cooperativity between CAMs has been observed at adherens junctions between nectins and integrins (Sakisaka *et al.*, 2007) and between nectins and cadherins at axo-dendritic contacts in hippocampal neuron cultures (Togashi *et al.*, 2006). So, does each CAM, be it a Nlgn, an EphB or a SynCAM molecule, contribute a small interaction to building the entire postsynaptic specialization or do they work together?

Is it possible that some CAMs synergize? Or are all their contributions small, thus making each individual CAM largely redundant.

Studies from knock-out mice lend weight to the hypothesis that, at least for the formation of synapses, synaptic CAMs are redundant. Removal of EphBs (Kayser *et al.*, 2008), neurexins (Missler *et al.*, 2003), Nlgns (Varoqueaux *et al.*, 2006) or SynCAM1 (Fujita *et al.*, 2006) does not abrogate the formation of synapses. However, the function of synapses in the adult animals is subtly altered. Neurexin knock-out mice present reduced release of synaptic vesicles due to impaired calcium entry (Missler *et al.*, 2003). Reduction of Nlgn1 expression in the amygdala reduces long term potentiation and results in a reduction in associative fear memory (Kim *et al.*, 2008). In knock-out Nlgn2 mice, inhibitory synaptic transmission from fast-spiking interneurons is reduced, while somatostatin-positive interneurons are spared (Gibson *et al.*, 2009). Furthermore, mutations in Nlgn genes (Jamain *et al.*, 2003; Laumonnier *et al.*, 2004) and in the SynCAM gene (CADM1; (Zhiling *et al.*, 2008) in humans may contribute to autism or mental retardation. While these disorders are traumatic, they underscore the fact that synapses do form and are still largely functional. We are still trying to grasp the subtle synaptic changes that might lead to the changes in behavior associated with autism. Thus, these observations would suggest that, for synaptic maintenance, the presence or absence of one specific CAM at a given developmental time point may fine-tune synapses. The key experiments to test the possible synergistic properties of synaptic CAMs remain to be performed. Crossing the mutant alleles for different CAM family members into a single mouse may start to shed light on this. However, interpretation of the resulting mice will be confounded by partially overlapping expression patterns; it will be necessary to concentrate on brain regions where the exact contribution of each CAM family member is well characterized. Perhaps, these experiments may have to first be confined to simpler systems, in which the assembly of an artificial postsynaptic density can be monitored and the individual or combined contributions of individual CAMs evaluated.

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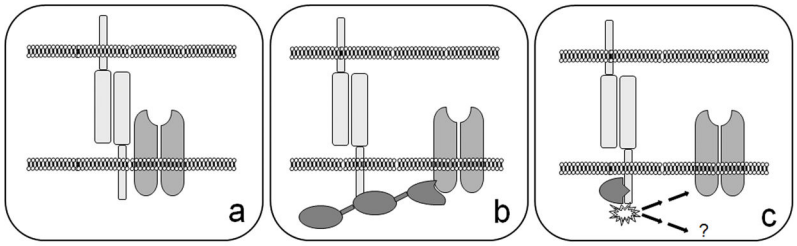

Figure 1.

Schematic demonstrating three mechanisms by which a trans-synaptic adhesion complex can stabilize NTRs at synapses: (a) a direct interaction between the CAMs and NTRs, (b) an indirect interaction between the CAM and the NTRs via a single or multiple scaffolding molecules and (c) trans-synaptic adhesion activates a signaling cascade that ultimately results in the recruitment or stabilization of NTRs at synapses.

Table 1

This table lists the known postsynaptic CAMs and their presynaptic ligands. The citation list is not exhaustive: citations list one of the more pertinent references supporting this interaction and/or its relevance to synaptic physiology.

Pre-	Post-	Citations
Cadherin	Cadherin	(Ranscht, 2000)
ephrinB	EphB	(Dalva *et al.*, 2000)
ECM?	integrin	(Chavis & Westbrook, 2001)
Neurexin	LRRTM	(de Wit *et al.*, 2009; Ko *et al.*, 2009)
Neurexin1	Neuroligin1	(Scheiffele *et al.*, 2000)
Reticulon3	SALM	(Chang *et al.*, 2010)
Sidekick2	Sidekick2	(Yamagata *et al.*, 2002)
SynCAM2	SynCAM1	(Biederer *et al.*, 2002; Fogel *et al.*, 2007)
netrin G	NGL2	(Kim *et al.*, 2006)
LAR	NGL3	(Woo *et al.*, 2009)

ECM, extracellular matrix; LAR, leukocyte common antigen-related.

Table 2

This table lists the scaffolding proteins that have been characterized to interact with synaptic CAMs via their PDZ binding motifs. PDZ binding motifs can be categorized as either Type I or Type II based on the nature of the −2 and 0 amino acids (see text). The three most C-terminal amino acids (−2, −1 and 0) of the murine protein sequences are reported here.

	CAM	PDZ motif	Interacting Proteins	Citations
Type I	LRRTM2	-CEV	PSD95	(Linhoff *et al.*, 2009)
	NGL2	-TQI	PSD95	(Kim *et al.*, 2006)
	Nlgn1	-TRV	PSD95, SAP102, Chapsyn110, MAGI1-3, Shank1, Shank3, Pick1, GOPC, SPAR, Semcap3, RGS3	(Irie *et al.*, 1997; Meyer *et al.*, 2004)
	Nlgn2	-TRV	PSD95, SAP102, Chapsyn110, MAGI1-3, Shank1, Shank3, Pick1, GOPC, SPAR, RGS3	(Meyer *et al.*, 2004)
	SALM2	-STV	PSD95 PSD95, SAP102, Chapsyn110, MAGI1-3, Shank	(Ko *et al.*, 2006)
	Sidekick2	-SFV	2, Shank3, Pick1, GOPC, SPAR, Semcap3, RGS3, scribble	(Meyer *et al.*, 2004)
Type II	EphB2	-VEV	Pick1, GRIP	(Torres *et al.*, 1998)
	EphB7	-IQV	Pick1, GRIP, syntenin	(Torres *et al.*, 1998)
	ephrinB1	-YKV	Pick1, GRIP, syntenin	(Torres *et al.*, 1998)
	ephrinB2	-YKV	PSD95, Pick1, SPAR, Semcap3, RGS3, GRIP, syntenin	(Meyer *et al.*, 2004)
	SynCAM1	-YFI	syntenin, GRIP*, CASK	(Biederer *et al.*, 2002; Meyer *et al.*, 2004; Hoy *et al.*, 2009)

* designates an interaction not validated in cultured mammalian cells.