ELSEVIER

Cell–cell interactions in synaptogenesis

Michael R Akins and Thomas Biederer

Synaptogenesis is a finely organized process, intriguing in its precise temporal and spatial resolution. It occurs as the dendrite of a postsynaptic neuron and an incoming axon communicate at defined sites to establish a stable synapse together. The molecular cues that guide synaptogenesis are now beginning to be identified, and cell surface interactions at synaptic sites participate prominently in the key steps. Interactions include trans-synaptic adhesion of pre- and post-synaptic neurons but also binding to non-neuronal neighboring cells and the extracellular matrix. These signals recruit scaffolding molecules, other adhesion molecules, and neurotransmitter receptors to bring together the key components of functional synapses. Recent progress provides stimulating insights into the role of adhesion and signaling molecules in the formation and function of synaptic specializations.

Addresses
Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, 333 Cedar Street, New Haven, CT, 06520, USA

Corresponding author: Biederer, Thomas (thomas.biederer@yale.edu)

Current Opinion in Neurobiology 2006, **16**:1–7

This review comes from a themed issue on
Development
Edited by Anirvan Ghosh and Christine E Holt

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2006.01.009

Introduction

Our understanding of synaptogenesis is rapidly advancing, and studies of cell surface molecules significantly contribute to this progress [1,2]. Adhesive interactions play roles in several stages of synaptogenesis, from the initial physical interaction of axons with target sites to subsequent synapse induction and later maturation steps. In this review, we focus on recent research highlighting the distinct functions of cell surface interactions during synaptogenesis in both vertebrate and invertebrate systems. We discuss new work demonstrating important roles for cell–cell adhesion molecules, the extracellular matrix and soluble signaling factors. Studies of cell surface interactions are likely to continue to yield important insights into the cellular and molecular events that guide synapse formation.

Cell–cell interactions in target recognition

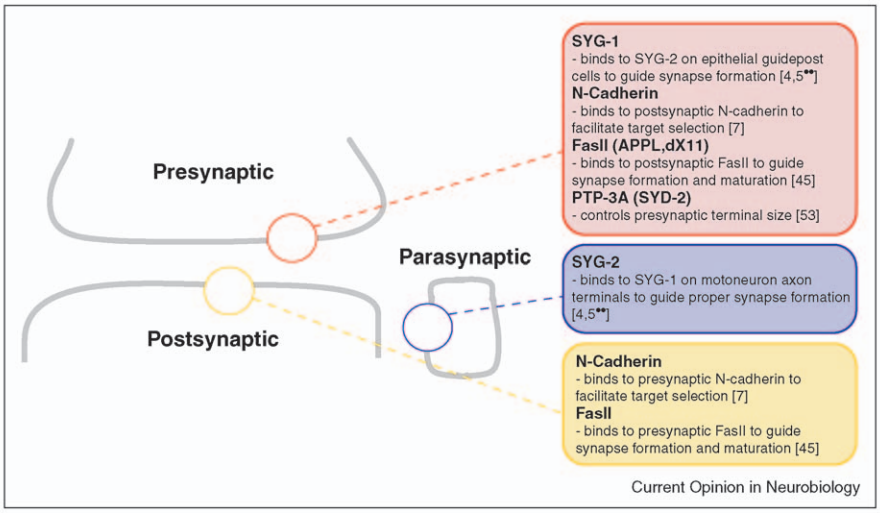
After axons and dendrites traverse considerable distances to reach their target, they are presented with an array of cells with which they could form synapses. Only a subset of these potential connections are actually formed and the subcellular localization of innervation is tightly regulated [3]. Important roles of intercellular adhesion molecules are being identified in these processes.

SYG-1 and SYG-2 are immunoglobulin (Ig)-superfamily members that were identified in *C. elegans* screens for mutants with improper placement of synapses on vulval muscle cells [4,5••] (see Figure 1 for invertebrate proteins active in synaptogenesis). Correct target recognition relies upon a heterophilic interaction between SYG-1 in the axons and SYG-2 in epithelial guidepost cells. In the absence of these molecular cues, the axons form ectopic synapses, suggesting that these proteins guide localization of, but not competency for, synapse formation. This implies that axons and dendrites identify the most appropriate target from a competitive landscape, rather than having a one-to-one relationship with a target cell. SYG-1 and -2 are homologous to the vertebrate nephrins, which have not been investigated for a role in synaptic target recognition. Their adhesive interactions might help to define patterns of synaptic innervation, similar to the function of Sidekick proteins in laminae of the retina [6].

N-cadherins have long been implicated in synaptic recognition. Support for this model comes from recent genetic studies in *Drosophila*, which demonstrate that N-cadherins on both photoreceptor cells and their target neurons in the optic neuropil are required for proper target selection [7]. Yet, in vertebrates, cadherins do not appear to function generally in target recognition [8] (see Figure 2 for vertebrate proteins active in synaptogenesis). Block of cadherin-dependent junction assembly does not interfere with synapse localization but rather with synapse maturation and function.

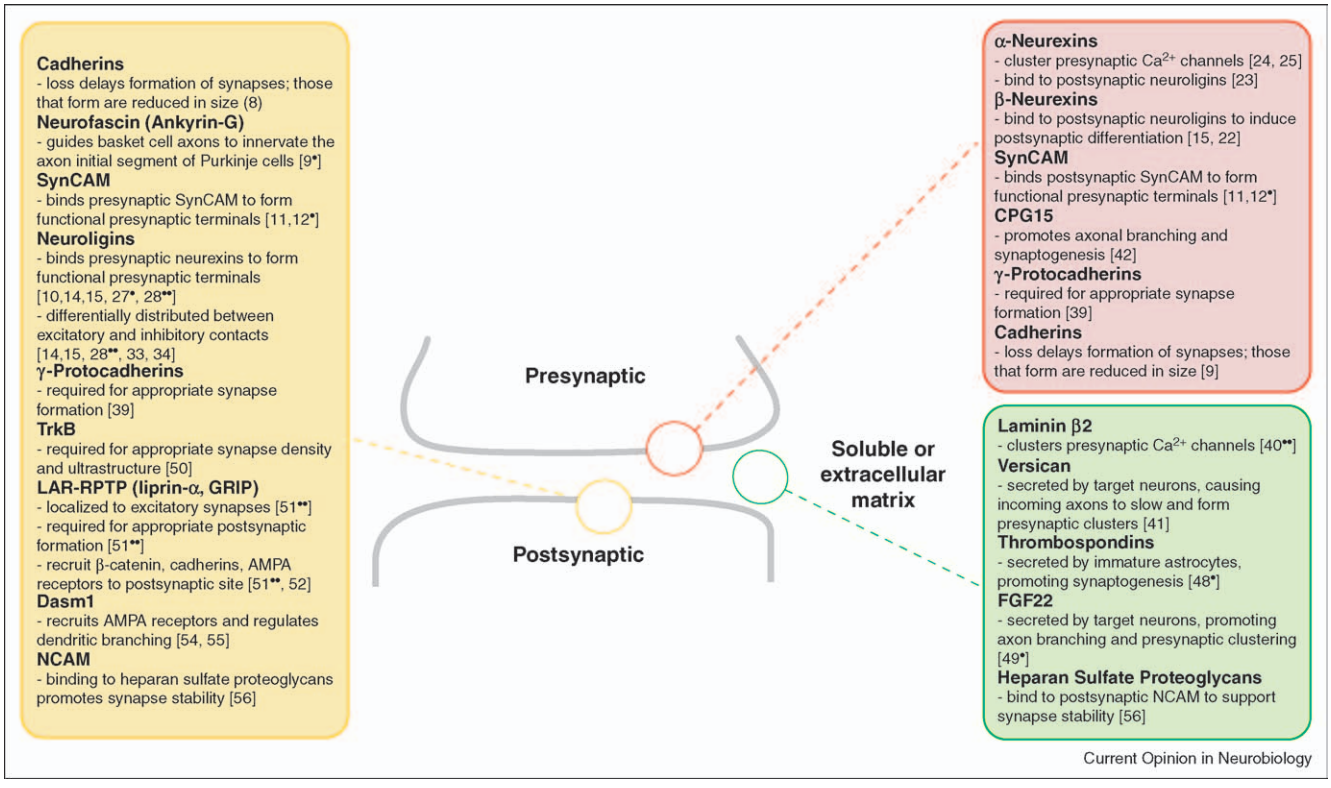
In addition to the selectivity for particular target neurons, certain classes of synaptic connections are segregated to particular subcellular compartments. A recent advance was made in understanding the molecular basis of subcellular target selection in the cerebellum. Here, neurofascin, a protein belonging to the L1 family of the Ig superfamily, specifies the site of synaptic input of basket neurons precisely onto the initial axon segment of Purkinje cells [9•]. This process additionally depends on the cytoskeletal adaptor protein ankyrin-G. In the absence of neurofascin and ankyrin-G, the correct number of synapses is not established. This is an important differ-

Figure 1



Summary of molecules at invertebrate synapses and their identified roles. Summary of molecules and their identified roles at invertebrate synapses. Recently characterized proteins include those expressed on presynaptic (red), postsynaptic (yellow), or parasynaptic (blue) membranes. No secreted or extracellular matrix molecules have yet been characterized to have a role in invertebrate synaptogenesis. Known intracellular interaction partners are indicated in parentheses.

Figure 2



Summary of molecules at vertebrate synapses and their identified roles. Recently characterized proteins include presynaptic (red), postsynaptic (yellow), and secreted or extracellular matrix (green) molecules. No non-neuronally expressed cell surface molecules have been identified to have a role in vertebrate synaptogenesis. Known intracellular interaction partners are indicated in parentheses.

ence from *C. elegans* lacking SYG proteins, wherein an appropriate number of synapses form ectopically. This could reflect a difference in the specific roles of these proteins or a general distinction between cues found on guidepost cells versus those on target cells.

Induction of synaptic specializations

Neuroligins and SynCAM 1 are the only adhesion molecules sufficient for the induction of presynaptic specialisations *in vitro* [10,11]. Notably, contact with these adhesion molecules induces neurons to assemble presynaptic terminals that have physiological properties virtually identical to those formed between neurons [12•]. Neuroligins and SynCAM 1 are localized at postsynaptic sites, and interact with presynaptic membrane proteins to exert their activity. SynCAM 1 engages in a trans-synaptic interaction with presynaptic SynCAM, and is a member of a vertebrate-specific family of four adhesion molecules [13]. Postsynaptic neuroligins heterophilically bind to the presynaptic neurexins to induce the formation of presynaptic terminals [10,14••,15]. Neuroligins are of importance in neurodevelopment: mutations in neuroligin genes that interfere with neuroligin trafficking to the cell surface [16,17] are linked to autism and mental retardation in humans [18,19]. This implies that altered synaptic differentiation plays a role in these disorders.

Neurexins are encoded by three different genes containing internal promoters, which give rise to variants with a short extracellular sequence, the β neurexins, and with a long extracellular sequence, the α neurexins [20]. Neuroligins can bind β neurexins, which led to their initial identification [21]. The β neurexin/neuroligin adhesion system is bi-directionally active: whereas neuroligin can induce presynaptic terminals, presynaptic β neurexin is capable of triggering postsynaptic assemblies through neuroligins [14••,22]. These β neurexin-induced dendritic clusters not only contain neuroligins and excitatory or inhibitory scaffolding molecules but also neurotransmitter receptors [14••,22]. They are likely to be immediate precursors for postsynaptic specializations. β neurexin is the first protein known to have this activity.

Unexpectedly, certain neuroligin variants bind not only β but also α neurexins [23]. This interaction is strictly regulated by one splice site present in neuroligins. α Neurexins have crucial presynaptic functions, and are necessary for correct function of presynaptic Ca^{2+} channels and release of neurotransmitters [24,25]. The same studies do not reveal a defect in synapse formation in absence of α neurexins. Trans-synaptic adhesion by the α neurexin/neuroligin system could, therefore, have important physiological roles beyond synapse formation, and modulate synaptic vesicle exocytosis.

Cytosolic interactions are crucial for the functions of the above-mentioned synapse-inducing molecules. Neuroligins, neurexins and SynCAM proteins all are single-spanning membrane proteins with intracellular carboxyl-terminal tails ending in consensus motifs predicted to bind PDZ-domain containing adaptor molecules. The SynCAM 1 cytosolic sequence is necessary for its activity to promote excitatory neurotransmission [12•] and PDZ-domain interactions are involved in SynCAM 1 effects on presynaptic terminal formation and function [11]. Multiple PDZ-domain containing proteins also can bind to the carboxyl-terminal motif of neuroligins [26], and PDZ domain interactions are likely to play a role in synaptic assembly. The adapter molecule PSD-95, located at excitatory postsynaptic sites, promotes clustering of neuroligin 1 and vice versa, and can recruit neuroligin 2 to excitatory synapses from inhibitory synapses, which is where endogenous neuroligin 2 is located [14••,15,27•,28••]. The synaptic localization of neuroligin family members is, therefore, more dynamic than previously thought. Intracellular sequences distinct from these PDZ interaction motifs are involved in the targeting of neuroligin to dendrites [29] and synapses [30,31].

Specification of synaptic connections

The initial induction of synapses, at least *in vitro*, is promiscuous, involving some degree of mismatch between excitatory and inhibitory pre- and post-synaptic components [32]. Mismatched sites are eliminated or converted until nearly all sites are correctly juxtaposed. The mechanisms mediating this refinement probably involve cell–cell interactions between apposed synaptic membranes.

Neuroligins play a role in this process in addition to their function in synapse induction. Neuroligin 1 is present only at excitatory postsynaptic sites, whereas neuroligin 2 is preferentially localized at inhibitory synapses [14••,15,28••,33,34], indicating potentially divergent functional roles in the specification of synaptic neurotransmitter type. Neuroligins 1, 2 and 3 promote formation of excitatory presynaptic terminals between neurons with similar activity [15,28••]. In addition, neuroligins promote inhibitory presynaptic terminal formation, with neuroligin 2 being the most active [15,27•,28••]. Neuroligins are also involved — in the reverse direction — in postsynaptic specification. Clustering of neuroligin 1 on dendritic surfaces leads to co-clustering of the excitatory postsynaptic scaffolding molecule PSD-95 at these sites. Neuroligin 2 clustering similarly causes local aggregation of PSD-95, but also of the inhibitory postsynaptic marker gephyrin [14••]. Concurrently, overexpression of neuroligins promotes dendritic spine formation, increases excitatory synapse density and recruitment of postsynaptic proteins, whereas neuroligin knockdown reduces excitatory and inhibitory synapse numbers [12•,15,28••]. The partial functional overlap of neuroligins in the induction of excitatory and inhibitory synapses probably explains why they appear redundant in knockdown experiments [28••]. This redundancy could involve the promiscuous

binding of presynaptic β neurexins to the different neuroligin proteins [35].

These studies clearly implicate neuroligins in synapse specification, yet do not identify a simple code that unambiguously determines induction of excitatory versus inhibitory synaptic specializations. The molecules identified to date to be involved in this process are all postsynaptic; by necessity, however, specifying information must be present on the presynaptic side as well. Thus, identification of adhesion molecules preferentially expressed in excitatory or inhibitory presynaptic membranes will be a major advance. These will be candidate proteins to participate alongside β-neurexins/neuroligins to achieve the precise pre- and post-synaptic match of neurotransmitter specificity found at mature synapses.

Among the molecules implicated in such a role are the protocadherins, a subset of the cadherin superfamily. Protocadherins comprise a large family, with nearly 60 members organized into three gene clusters [36]. Enriched at synapses and with a large number of potentially specific binding partners, α- and γ-protocadherins are well suited to specify appropriate synaptic interactions in distinct, although overlapping, subsets of neurons [37,38]. Indeed, loss of the γ-protocadherin gene cluster leads to synaptic deficits in mouse spinal cord [39], with a reduction in the number of both excitatory and inhibitory puncta and a concomitant loss in excitatory puncta size. It remains to be determined whether individual γ-protocadherins specify identifiable sets of synapses.

Presynaptic interactions in synapse maturation and stabilization

An important advance in our understanding of presynaptic maturation was made at the neuromuscular junction. The laminin β2 subunit, a component of the extracellular matrix within the synaptic cleft of neuromuscular synapses, binds to presynaptic voltage-gated Ca^{2+} channels [40••]. This interaction clusters these channels and is required for correct active zone assembly. This study highlights the important roles of extracellular matrix interactions in synaptic differentiation, which can also be expected to affect formation of synapses in the CNS. Indeed, the proteoglycan versican supports the maturation of synaptic contacts of incoming retinal axons, target neurons of which are in laminae of the optic tectum [41]. Versican is a component of the extracellular matrix surrounding these target neurons, and the presynaptic terminals of innervating retinal axons increase their size upon contact. Hence, versican combines two important functions — target recognition and presynaptic maturation.

Interestingly, the same signaling pathway can mediate both presynaptic differentiation and axonal morphological changes. The activity-induced membrane protein CPG15 promotes axon branching and extension in addition to synapse formation when overexpressed in axons [42]. Notably, this study showed that new axon branches emerge from presynaptic sites, indicating that both are formed by overlapping differentiation pathways. Integrin binding to the extracellular matrix and ensuing signaling might participate in these differentiation events, as the integrin-activated nonreceptor tyrosine kinase FAK (focal adhesion kinase) downregulates axon branching and synapse number [43].

A prominent invertebrate Ig-domain containing protein with a role in synapse stabilization and maturation is the *Drosophila* FasII protein. A widely held model for the role of FasII is that expression of the molecule constrains synaptogenesis, whereas hypomorphic expression removes this restriction. This model is supported by studies in which FasII expression levels were lowered or raised on one side of the fly neuromuscular junction [44]. However, raising expression levels of FasII on both sides of the synapse simultaneously results in an increase in synaptic bouton number [45], calling for a refinement of this model. The morphological and physiological changes elicited by altering FasII levels depend on its ability to bind the *Drosophila* homolog of the amyloid precursor protein, APPL, and the scaffolding protein Mint 1. Interestingly, the deletion of APP and APP-like protein 2 in mice causes multiple ultrastructural presynaptic defects [46,47], indicating that they organize presynaptic terminals in both vertebrates and invertebrates. The importance of symmetric expression of a homophilic adhesion molecule across the synaptic cleft, as shown for FasII, indicates that appropriately matched levels of signaling and scaffolding molecules need to be recruited to both sides of a nascent synapse. Thus, an important determinant of synapse number and location might well be not only the identity of cell adhesion molecules on the apposed membranes, but also their local concentration.

Several soluble factors are now known that control presynaptic differentiation [48•,49•]. Thrombospondins (TSPs), extracellular matrix molecules secreted by astrocytes, promote formation of synapses *in vitro* and TSP knockout animals display reduced synapse density [48•]. TSPs affect presynaptic properties and are likely to stabilize newly forming synaptic sites, highlighting the roles of glial cells in synapse development. By contrast, the fibroblast growth factor family member FGF-22 is secreted by target neurons [49•]. FGF-22 promotes clustering of recycling synaptic vesicles in terminals that form on cerebellar granule cells and enhances axon branching. It is likely to function through a presynaptic or axonal FGF receptor. TSP and FGF signaling might operate in multiple types of central neurons, but this remains to be elaborated.

Involvement of the postsynaptic side in synaptic differentiation

Postsynaptic signaling through the neurotrophin receptor TrkB is necessary for correct synapse density, ultrastruc-

ture and excitatory neurotransmission [50]. Other cell surface molecules have also been linked to the maturation of postsynaptic excitatory sites. The leukocyte common antigen-related family receptor protein tyrosine phosphatases (LAR-RPTP) proteins have extracellular Ig and fibronectin domains similar to adhesion molecules and are localized to excitatory synapses in the mammalian nervous system [51••]. LAR-RPTPs interact intracellularly with liprin-α and the adapter molecule GRIP to recruit AMPA receptors to synapses and affect postsynaptic maturation [52]. Interference with the phosphatase activity of LAR-RPTPs or their ability to interact with liprin-α decreases synapse and spine density and excitatory neurotransmission [51••]. This process involves the interaction between liprin-α and GRIP downstream of LAR-RPTPs. These effects probably reflect a role of LAR-RPTPs in recruitment of other proteins to nascent synapses, notably proteins involved in cadherin junctions. As block of cadherin function reduces synapse size, vesicle recycling and frequency of excitatory neurotransmission [8], it appears that the LAR-RPTPs and cadherins function together. LAR not only organizes maturing postsynaptic sites but also affects presynaptic active zone differentiation. In *C. elegans*, the LAR family member PTP-3A anchors the liprin-α homolog SYD-2 at presynaptic sites and thus controls presynaptic terminal size [53].

Dasm1, a recently identified Ig-domain containing protein, also functions in postsynaptic differentiation [54,55]. Postsynaptic Dasm1 recruits AMPA receptors and affects excitatory transmission through intracellular interactions with PDZ domain containing proteins [54]. Dasm1, in addition, modulates the outgrowth and complexity of dendritic arbors, indicating that dendritic and postsynaptic differentiation can utilize the same pathways [55], similar to how presynaptic and axonal differentiation can use the same pathways. The presynaptic interaction partner of Dasm1 is yet unknown. New evidence for a role of a different Ig-superfamily member, NCAM, in synaptic differentiation further implicates extracellular matrix interactions in synaptic differentiation [56]. The postsynaptic expression of NCAM and its binding of proteoglycans is correlated with enhanced synapse formation.

Conclusions and outlook

The analysis of cell surface interactions guiding synaptogenesis is burgeoning at a rapid pace with the identification of more players. This advance is likely to continue. The extracellular interaction partners for several of the molecules reviewed here remain to be identified. Similarly, the role of the extracellular matrix in synapse induction and maturation is likely to receive more attention. On the intracellular side of these interactions, many of the signaling pathways and protein scaffolds that develop at sites of nascent synapses remain to be unraveled. Furthermore, functional studies of synaptic differentiation will benefit from analyses of concomitant structural changes. This will be necessary to understand the spatially extremely well defined assembly of synaptic membranes. An important step into this direction is the recent high-resolution visualization of adhesive interactions in the synaptic cleft [57]. Finally, the analysis of known cell–cell interactions in synaptogenesis will need to include more *in vivo* studies to complement the current focus on results obtained in neuronal culture. Genetic screens for mutants in this process, for example in zebrafish [58], might be one *in vivo* approach to identify novel vertebrate molecules participating in this process.

Acknowledgements

We apologize for omitted citations due to space limitations. Work in our laboratory is supported by the National Institutes of Health Grant RO1 DA018928, The Brain Tumor Society, and the March of Dimes Foundation Grant 5-FY2005-138.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Waites CL, Marie Craig A, Garner CC: **Mechanisms of vertebrate synaptogenesis**. *Annu Rev Neurosci* 2005, **28**:251-274.

2. Li Z, Sheng M: **Some assembly required: The development of neuronal synapses**. *Nat Rev Mol Cell Biol* 2003, **4**:833-841.

3. Somogyi P, Tamas G, Lujan R, Buhl EH: **Salient features of synaptic organisation in the cerebral cortex**. *Brain Res Brain Res Rev* 1998, **26**:113-135.

4. Shen K, Bargmann CI: **The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans***. *Cell* 2003, **112**:619-630.

5. •• Shen K, Fetter RD, Bargmann CI: **Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1**. *Cell* 2004, **116**:869-881.
The authors identify here and in Shen and Bargmann [4] that the two Ig-superfamily members SYG-1 and -2 operate together in *C. elegans* to specify the site of innervation on locally defined muscles. This study provides detailed insights into how a specific heterophilic adhesive interaction can guide synapse formation.

6. Yamagata M, Weiner JA, Sanes JR: **Sidekicks: Synaptic adhesion molecules that promote lamina-specific connectivity in the retina**. *Cell* 2002, **110**:649-660.

7. Prakash S, Caldwell JC, Eberl DF, Clandinin TR: **Drosophila N-cadherin mediates an attractive interaction between photoreceptor axons and their targets**. *Nat Neurosci* 2005, **8**:443-450.

8. Bozdagi O, Valcin M, Poskanzer K, Tanaka H, Benson DL: **Temporally distinct demands for classic cadherins in synapse formation and maturation**. *Mol Cell Neurosci* 2004, **27**:509-521.

9. • Ango F, di Cristo G, Higashiyama H, Bennett V, Wu P, Huang ZJ: **Ankyrin-based subcellular gradient of neurofascin, an immunoglobulin family protein, directs GABAergic innervation at Purkinje axon initial segment**. *Cell* 2004, **119**:257-272.
This study reveals that a gradient of the Ig-superfamily member neurofascin186 marks subcellular sites of synapse formation, a process of crucial physiological importance for the establishment of neuronal circuits.

10. Scheiffele P, Fan J, Choih J, Fetter R, Serafini T: **Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons**. *Cell* 2000, **101**:657-669.

11. Biederer T, Sara Y, Mozhayeva M, Atasoy D, Liu X, Kavalali ET, Südhof TC: **SynCAM, a synaptic adhesion molecule that drives synapse assembly**. *Science* 2002, **297**:1525-1531.

12. • Sara Y, Biederer T, Atasoy D, Chubykin A, Mozhayeva MG, Südhof TC, Kavalali ET: **Selective capability of SynCAM and neuroligin for functional synapse assembly**. *J Neurosci* 2005, **25**:260-270.
The authors identify that SynCAM 1 and neuroligin 1 induce fully functional presynaptic terminals that engage in evoked and miniature responses, as shown in a reconstituted neurotransmission system.

13. Biederer T: **Bioinformatic characterization of the SynCAM family of immunoglobulin-like domain containing adhesion molecules**. *Genomics* 2006, **87**:139-150.

14. •• Graf ER, Zhang X, Jin S-X, Linhoff MW, Craig AM: **Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins**. *Cell* 2004, **119**:1013-1026.
This work establishes that β neurexin, through its interaction with postsynaptic neuroligins, induces local clustering of postsynaptic markers characteristic for excitatory or inhibitory synapses. This is the first study to identify molecular signals in the formation of postsynaptic specializations, and delineates differences among neuroligin proteins in this process.

15. Levinson JN, Chery N, Huang K, Wong TP, Gerrow K, Kang R, Prange O, Wang YT, El-Husseini A: **Neuroligins mediate excitatory and inhibitory synapse formation: involvement of PSD-95 and neurexin-1beta in neuroligin-induced synaptic specificity**. *J Biol Chem* 2005, **280**:17312-17319.

16. Chih B, Afridi SK, Clark L, Scheiffele P: **Disorder-associated mutations lead to functional inactivation of neuroligins**. *Hum Mol Genet* 2004, **13**:1471-1477.

17. Chubykin AA, Liu X, Comoletti D, Tsigelny I, Taylor P, Sudhof TC: **Dissection of synapse induction by neuroligins: effect of a neuroligin mutation associated with autism**. *J Biol Chem* 2005, **280**:22365-22374.

18. Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C *et al.*: **Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism**. *Nat Genet* 2003, **34**:27-29.

19. Laumonnier F, Bonnet-Brilhault F, Gomot M, Blanc R, David A, Moizard MP, Raynaud M, Ronce N, Lemonnier E, Calvas P *et al.*: **X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family**. *Am J Hum Genet* 2004, **74**:552-557.

20. Ushkaryov YA, Petrenko AG, Geppert M, Südhof TC: **Neurexins: synaptic cell surface proteins related to the alpha-latrotoxin receptor and laminin**. *Science* 1992, **257**:50-56.

21. Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Südhof TC: **Neuroligin 1: a splice site-specific ligand for beta-neurexins**. *Cell* 1995, **81**:435-443.

22. Nam CI, Chen L: **Postsynaptic assembly induced by neurexin-neuroligin interaction and neurotransmitter**. *Proc Natl Acad Sci USA* 2005, **102**:6137-6142.

23. Boucard AA, Chubykin AA, Comoletti D, Taylor P, Sudhof TC: **A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins**. *Neuron* 2005, **48**:229-236.

24. Missler M, Zhang WQ, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K, Südhof TC: **Alpha-neurexins couple Ca2+ channels to synaptic vesicle exocytosis**. *Nature* 2003, **423**:939-948.

25. Zhang W, Rohlmann A, Sargsyan V, Aramuni G, Hammer RE, Sudhof TC, Missler M: **Extracellular domains of alpha-neurexins participate in regulating synaptic transmission by selectively affecting N- and P/Q-type Ca2+ channels**. *J Neurosci* 2005, **25**:4330-4342.

26. Meyer G, Varoqueaux F, Neeb A, Oschlies M, Brose N: **The complexity of PDZ domain-mediated interactions at glutamatergic synapses: a case study on neuroligin**. *Neuropharmacology* 2004, **47**:724-733.

27. • Prange O, Wong TP, Gerrow K, Wang YT, El-Husseini A: **A balance between excitatory and inhibitory synapses is controlled by PSD-95 and neuroligin**. *Proc Natl Acad Sci USA* 2004, **101**:13915-13920.
The authors discover, together with Chih *et al*. [28••], that the β-neurexin/neuroligin interaction induces not only excitatory but also inhibitory presynaptic terminals, and characterizes functions of the intracellular adaptor molecule PSD-95 in this process.

28. •• Chih B, Engelman H, Scheiffele P: **Control of excitatory and inhibitory synapse formation by neuroligins**. *Science* 2005, **307**:1324-1328.
Using overexpression and knockdown in culture, the authors demonstrate that neuroligins mediate formation of both excitatory and inhibitory presynaptic terminals. This study shows that neuroligin 2 is more active in the induction of inhibitory presynaptic terminals than the other neuroligins, providing insights into the interactions that help to define neurotransmitter specificity of central synapses.

29. Rosales CR, Osborne KD, Zuccarino GV, Scheiffele P, Silverman MA: **A cytoplasmic motif targets neuroligin-1 exclusively to dendrites of cultured hippocampal neurons**. *Eur J Neurosci* 2005, **22**:2381-2386.

30. Dresbach T, Neeb A, Meyer G, Gundelfinger ED, Brose N: **Synaptic targeting of neuroligin is independent of neurexin and SAP90/PSD95 binding**. *Mol Cell Neurosci* 2004, **27**:227-235.

31. Iida J, Hirabayacshi S, Sato Y, Hata Y: **Synaptic scaffolding molecule is involved in the synaptic clustering of neuroligin**. *Mol Cell Neurosci* 2004, **27**:497-508.

32. Anderson TR, Shah PA, Benson DL: **Maturation of glutamatergic and GABAergic synapse composition in hippocampal neurons**. *Neuropharmacology* 2004, **47**:694-705.

33. Song JY, Ichtchenko K, Südhof TC, Brose N: **Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses**. *Proc Natl Acad Sci USA* 1999, **96**:1100-1105.

34. Varoqueaux F, Jamain S, Brose N: **Neuroligin 2 is exclusively localized to inhibitory synapses**. *Eur J Cell Biol* 2004, **83**:449-456.

35. Ichtchenko K, Nguyen T, Südhof TC: **Structures, alternative splicing, and neurexin binding of multiple neuroligins**. *J Biol Chem* 1996, **271**:2676-2682.

36. Wu Q, Maniatis T: **A striking organization of a large family of human neural cadherin-like cell adhesion genes**. *Cell* 1999, **97**:779-790.

37. Phillips GR, Tanaka H, Frank M, Elste A, Fidler L, Benson DL, Colman DR: **Gamma-protocadherins are targeted to subsets of synapses and intracellular organelles in neurons**. *J Neurosci* 2003, **23**:5096-5104.

38. Frank M, Ebert M, Shan W, Phillips GR, Arndt K, Colman DR, Kemler R: **Differential expression of individual gamma-protocadherins during mouse brain development**. *Mol Cell Neurosci* 2005, **29**:603-616.

39. Weiner JA, Wang X, Tapia JC, Sanes JR: **Gamma protocadherins are required for synaptic development in the spinal cord**. *Proc Natl Acad Sci USA* 2005, **102**:8-14.

40. •• Nishimune H, Sanes JR, Carlson SS: **A synaptic laminin-calcium channel interaction organizes active zones in motor nerve terminals**. *Nature* 2004, **432**:580-587.
The authors identify the direct binding of presynaptic voltage-gated Ca^{2+} channels to laminin β2 at the neuromuscular junction. This interaction is necessary to maintain active zones in presynaptic motor neuron terminals in a process guided by the basal lamina at the synaptic cleft.

41. Yamagata M, Sanes JR: **Versican in the developing brain: lamina-specific expression in interneuronal subsets and role in presynaptic maturation**. *J Neurosci* 2005, **25**:8457-8467.

42. Javaherian A, Cline HT: **Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 *in vivo***. *Neuron* 2005, **45**:505-512.

43. Rico B, Beggs HE, Schahin-Reed D, Kimes N, Schmidt A, Reichardt LF: **Control of axonal branching and synapse formation by focal adhesion kinase**. *Nat Neurosci* 2004, **7**:1059-1069.

44. Schuster CM, Davis GW, Fetter RD, Goodman CS: **Genetic dissection of structural and functional components of**

synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. *Neuron* 1996, **17**:641-654.

45. Ashley J, Packard M, Ataman B, Budnik V: **Fasciclin II signals new synapse formation through amyloid precursor protein and the scaffolding protein dX11/Mint**. *J Neurosci* 2005, **25**:5943-5955.

46. Yang G, Gong YD, Gong K, Jiang WL, Kwon E, Wang P, Zheng H, Zhang XF, Gan WB, Zhao NM: **Reduced synaptic vesicle density and active zone size in mice lacking amyloid precursor protein (APP) and APP-like protein 2**. *Neurosci Lett* 2005, **384**:66-71.

47. Wang P, Yang G, Mosier DR, Chang P, Zaidi T, Gong YD, Zhao NM, Dominguez B, Lee KF, Gan WB *et al.*: **Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-Like protein 2**. *J Neurosci* 2005, **25**:1219-1225.

48. • Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA: **Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis**. *Cell* 2005, **120**:421-433.
The authors show that thrombospondins are secreted, glia-derived proteins affecting neuronal and synaptic differentiation, underscoring the importance of glia–neuron interactions for synaptogenesis.

49. • Umemori H, Linhoff MW, Ornitz DM, Sanes JR: **FGF22 and its close relatives are presynaptic organizing molecules in the mammalian brain**. *Cell* 2004, **118**:257-270.
Employing activity-based biochemical purification, the authors identify FGF-22 as a secreted protein that affects both axonal and presynaptic differentiation.

50. Luikart BW, Nef S, Virmani T, Lush ME, Liu Y, Kavalali ET, Parada LF: **TrkB has a cell-autonomous role in the establishment of hippocampal Schaffer collateral synapses**. *J Neurosci* 2005, **25**:3774-3786.

51. •• Dunah AW, Hueske E, Wyszynski M, Hoogenraad CC, Jaworski J, Pak DT, Simonetta A, Liu G, Sheng M: **LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory synapses**. *Nat Neurosci* 2005, **8**:458-467.
Using overexpression, knockdown and dominant-negative approaches, the authors identify that the membrane-bound receptor tyrosine phosphatase LAR-RPTP affects the formation of excitatory synapses. This activity regulates postsynaptic recruitment and tyrosine phosphorylation of β-catenin. This study provides insights into signaling mechanisms during synaptogenesis.

52. Wyszynski M, Kim E, Dunah AW, Passafaro M, Valtschanoff JG, Serra-Pages C, Streuli M, Weinberg RJ, Sheng M: **Interaction between GRIP and liprin-alpha/SYD2 is required for AMPA receptor targeting**. *Neuron* 2002, **34**:39-52.

53. Ackley BD, Harrington RJ, Hudson ML, Williams L, Kenyon CJ, Chisholm AD, Jin Y: **The two isoforms of the *Caenorhabditis elegans* leukocyte-common antigen related receptor tyrosine phosphatase PTP-3 function independently in axon guidance and synapse formation**. *J Neurosci* 2005, **25**:7517-7528.

54. Shi SH, Cheng T, Jan LY, Jan YN: **The immunoglobulin family member dendrite arborization and synapse maturation 1 (Dasm1) controls excitatory synapse maturation**. *Proc Natl Acad Sci USA* 2004, **101**:13346-13351.

55. Shi SH, Cox DN, Wang D, Jan LY, Jan YN: **Control of dendrite arborization by an Ig family member, dendrite arborization and synapse maturation 1 (Dasm1)**. *Proc Natl Acad Sci USA* 2004, **101**:13341-13345.

56. Dityatev A, Dityateva G, Sytnyk V, Delling M, Toni N, Nikonenko I, Muller D, Schachner M: **Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses**. *J Neurosci* 2004, **24**:9372-9382.

57. Lucic V, Yang T, Schweikert G, Forster F, Baumeister W: **Morphological characterization of molecular complexes present in the synaptic cleft**. *Structure (Camb)* 2005, **13**:423-434.

58. Panzer JA, Gibbs SM, Dosch R, Wagner D, Mullins MC, Granato M, Balice-Gordon RJ: **Neuromuscular synaptogenesis in wild-type and mutant zebrafish**. *Dev Biol* 2005.