Published in final edited form as:

Curr Opin Neurobiol. 2011 February ; 21(1): 93–99. doi:10.1016/j.conb.2010.10.004.

Genetic Dissection of Synaptic Specificity

Celine I. Maeder¹ and Kang Shen^{1,2,3}

¹Department of Biology, Stanford University, Stanford, CA 94305, USA

²Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA

³Department of Pathology, Stanford University, Stanford, CA 94305, USA

Abstract

Nervous systems are built of a myriad of neurons connected by an even larger number of synapses. While it has been long known that neurons specifically select their synaptic partners among many possible choices during development, we only begin to understand how they make those decisions. Recent findings have started to elucidate the molecular mechanisms underlying synaptic target selection including positive as well as negative cues from synaptic partners, intermediate targets and surrounding tissues. Furthermore, emerging evidence suggests that synaptic connections are not only formed among specific sets of neurons, but they are also targeted to specific subcellular domains. Finally, spatial and temporal transcriptional regulation of these molecular cues represents an additional, versatile mechanism to provide wiring specificity.

Introduction

Neuronal circuit formation encompasses many interrelated developmental processes such as cell fate determination, cell migration and polarization, axon guidance, synaptic target selection, synapse formation and maturation. Emerging evidence suggests that neurons not only choose their synaptic partners, but also specifically direct synapse formation onto subcellular compartments of their partners.

In this review we will mainly focus on molecular mechanisms underlying cellular and subcellular synapse specificity. In particular we will highlight molecular cues such as cell adhesion molecules and morphogenetic gradients, which are present at or secreted from pre- or postsynaptic partners, guidepost cells and surrounding tissue. We will also discuss how transcriptional regulation of synaptic cues acts as an additional mechanism to control synaptic specificity.

Target cell selection

Mutual attractive recognition through cell adhesion/cell surface molecules

One popular conceptual model for synaptic specificity is a direct recognition between the pre- and postsynaptic partners through their complementary set of cell adhesion molecules presented across the synaptic cleft. Interactions between these adhesion molecules, often in a

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding Author: Kang Shen, Ph.D., 385 Serra Mall, Herrin Labs, Stanford University, Stanford, CA 94305-5020, USA, Tel: (650)724-4255, Fax: (650)725-4309, kangshen@stanford.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

homotypic fashion, are thought to promote the connectivity between synaptic partners. In the vertebrate retina, retinal interneurons synapse onto retinal ganglion cells (RGCs) in a highly ordered laminar arrangement in the inner plexiform layer (IPL). Yamagata and coworkers showed that immunoglobulin-superfamily (IgSF) proteins Sidekick-1, Sidekick-2, Dscam and DscamL are expressed in subpopulations of RGCs and interneurons, and that these molecules exclusively engage in homophilic interactions. Furthermore, pre- and postsynaptic partner cells, which express the same IgSF protein, form synapses in the same retinal sublamina within the IPL [1,2]. Thus, an IgSF code appears to dictate laminar specificity in the vertebrate retina.

In a similar manner, the leucine-rich repeat (LRR) proteins Capricious (Caps) and Tartan are expressed in specific presynaptic neurons and their postsynaptic targets in *Drosophila*. Loss of function or ectopic expression of Caps and Tartan alter the synaptic target selection [3–6]. Interestingly, a recent study showed a synaptic targeting function of Caps and Tartan in the fly olfactory system. However, in this system their functions do not appear to be dependent on homophilic interactions [7]. Several other types of synaptic adhesion molecules including the intensely studied Neurexin/Neurologin [8], Eph/ephrin [9] and SynCAM [10], are capable of inducing synapse formation *in vitro*. Genetic analyses argue that their *in vivo* functions are more likely to modify the strength of synaptic connections and to regulate the number of synapses. Whether they participate in the process of synaptic partner selection is yet not explored.

Repulsive cues fine-tune synaptic target selection

Up to date most molecules implicated in synaptic specificity act as attractive cue (Table 1). However, it is becoming increasingly clear that inhibitory cues from inappropriate targets might fine-tune synaptic target selection.

In a recent publication Pecho-Vrieseling and co-workers demonstrated that matching expression of Sema3e and Plxnd1 determines synaptic specificity in sensory-motor reflex arcs in mice [11]. In the cutaneous maximus (Cm) reflex arc, Cm motor neurons (MNs) lack input from Cm afferents. In contrast, in the triceps (tri) reflex arc, tri MNs receive monosynaptic input from tri afferents. While most afferents of both reflex arcs express Plxnd1, only Cm MNs express Sema3e. Loss of Sema3a or Plxnd1 by Cm MN or Cm afferents, respectively, leads to inappropriate monosynaptic circuit assembly. Inversely, ectopic expression of Sema3a in tri MN inhibits its synapse formation with tri afferents.

Another study at *Drosophila* neuromuscular junction (NMJ) identified Wnt4 as a local inhibitory cue [12] (Figure 1a). Neighboring muscles M12 and M13 are innervated by MN12s and RP1/4 neurons respectively. Inaki et al. identified Wnt4 being expressed by M13 but not M12. Wnt4 loss-of-function mutants displayed inappropriate synapse formation of MN12s onto M13, while ectopic expression of Wnt4 in M12 inhibited MN12s synapses. In the same NMJ system, the LRR protein Toll is expressed on non-target cells to repress synapse formation between improper partner cells [13] (Figure 1a).

Morphogenetic gradients as determinants of synaptic specificity

Many morphogens have been shown to play important roles in topographic map establishment, axon guidance and synaptogenesis [14]. Emerging evidence now also implicates these molecules in synaptic target selection.

The axons of dentate gyrus granule cells (GCs), also known as hippocampal mossy fibers, form large synaptic boutons onto pyramidal neurons in CA3. Galimberti et al. showed in a recent study that there are subpopulations of mossy fibers forming 0, 1 or more than 2 extremely large synaptic boutons, also called terminal arborizations (TAs) [15]. The position

of these TAs along CA3 shows a topographic distribution based on the cell body position of GCs in dentate gyrus, and it is dependent on Eph4A signaling and its graded expression in the dentate gyrus.

In the fly antennal lobe olfactory receptor neurons (ORNs) expressing a specific olfactory receptor project their axons to the same glomerulus [16]. Projection neurons (PN), postsynaptic partner cells of the ORNs, independently project their dendrites in a spatially defined map to the antennal lobe before ORNs axon innervation occurs [17]. Komiyama et al. showed that a graded expression of Semaphorin1a by PNs is responsible for proper dendritic targeting within the antennal lobe [18].

The role of guidepost cells in synaptic match-making

An extensive body of work has demonstrated that intermediate targets play a crucial role in proper axon guidance [19]. Recently, several studies have implicated guidepost cells such as glia in synaptic match-making.

In the cerebellum, Purkinje neurons receive at least four sets of subcellularly restricted inputs. Stellate interneurons mainly innervate the distal part of the Purkinje dendritic arbor. Ango et al. showed that stellate axons are guided towards Purkinje dendrites by intermediate target cells, the Bergmann glia [20] (Figure 1b). Furthermore they identified the L1 family immunoglobulin protein Close Homologue of L1 (CHL1) as an essential cue on Bergmann glia fibers for proper stellate axon innervation onto Purkinje dendrites.

Similar evidence for glia as guidepost cells in synaptic target selection comes from work in *C.elegans*. AIY interneuron forms synapses onto RIA interneuron, and accurate synapse formation depends on netrin signaling [21]. Interestingly, netrin is secreted by glia-like sheath cells, which adjoin the synaptic area of AIY to RIA. Netrin induces distinct UNC-40/DCC-dependent mechanisms in the two interneurons. In RIA, netrin signaling regulates axon guidance, while in AIY it is important for accurate synapse localization.

A third example for intermediate targets in synaptic match-making comes from studies on the egg-laying motor neuron HSNL in *C.elegans* [22,23]. HSNL forms synapses with VC neurons and the vulval muscle. Surprisingly, the subcellular localization of HSNL synapses is dictated by neighboring vulval epithelium cells and not the postsynaptic partners. The heterophilic interaction of two IgSF proteins, SYG-1 and SYG-2 expressed by HSNL and epithelial cells respectively, instruct the precise subcellular synaptic localization. In the absence of SYG-1 or SYG-2, HSNL still forms synapses but with inappropriate partners.

Synapse elimination tightens synaptic specificity

During nervous system development, excess innervation occurs, which is reduced later on during nervous system maturation and therefore renders synaptic connections more precise. While it is well known that neuronal activity is critical to shape synaptic circuit by modulating synapse elimination, molecular cues involved in synapse elimination have just started to be discovered.

In the hippocampus, dentate gyrus GC innervate CA3 pyramidal cells through two types of mossy fibers: the permanent main bundle projecting onto CA3 apical dendrites, and the infrapyramidal bundle establishing synapses onto CA3 basal dendrites. At early postnatal stages infrapyramidal bundle axons form transient connections that are eliminated at later time points of development [24]. Liu and coworkers further provide compelling evidence that PlxA3 and Npn2 signaling is indispensable for these processes.

Synapse elimination also plays an important role in the maturation of visual circuits. Initially, dorsal lateral geniculate nucleus (dLGN) neurons are innervated by multiple RGCs, but upon circuit maturation only one or two RGCs innervate the same dLGN neuron due to ample synapse elimination [25]. Stevens et al. showed that classical complement cascade proteins (C1q and C3) are upregulated by RGCs during visual circuit maturation and that they are essential for synapse pruning [26].

Little is known about how extrinsic cues lead to the disassembly of specific synapses while others are maintained. One study in *C.elegans* identified the ubiquitin degradation system important for selective synapse elimination [27]. In HSNL neuron, the anterior-most synapses are eliminated at L4 larval stage to yield the mature synaptic pattern. Only synapses, which colocalize with SYG-1 are maintained. Using a yeast two-hybrid assay, Ding et al. identified SKR-1, a component of the SKP-Cullin-F-box ubiquitin ligase complex, as an interacting partner of SYG-1. Interestingly, SYG-1 binding to SKR-1 disrupts the ubiquitin ligase complex thereby protecting SYG-1 positive synapses from elimination.

Subcellular specificity

Subcellular specificity targets synapse formation to a precise location on the postsynaptic partner, such as dendritic spine, dendritic shaft, axon initial segment (AIS) or cell soma. This subcellular targeting has been shown to profoundly impact on the properties of synaptic circuits [28,29].

Subcellular synapse targeting through cell adhesion molecules

In the visual cortex, subcellular targeting of distinct GABAergic inputs onto pyramidal cells appears to be independent of thalamic input and experience, pointing to a genetically encoded mechanism [30]. Notably, Purkinje cells in the cerebellum are specifically innervated at the AIS by basket cells. Ango and coworkers demonstrated that this precise innervation pattern depends on a gradient of neurofascin186 (NF186) on Purkinje cells, which is highest at AIS and decreases towards the soma and which, in turn, depends on AnkyrinG [31] (Figure 1b). Genetic ablation of NF186 gradient leads to aberrant local axon targeting and much reduced synapse formation.

Another well-studied example for subcellular synapse specificity comes from the vertebrate NMJ. Mutual signaling between motoneurons and muscles dictates NMJ formation only at the middle of muscle bands. Muscles are prepatterned through Musk-dependent (muscle-specific receptor tyrosine kinase) neuronal acetylcholine receptor (AChR) clustering before innervation [32,33]. Ingrowing motor axons then secrete agrin, which binds to postsynaptic Lrp4 receptor leading to the activation of MuSK-rapsyn downstream signaling and consecutive enhanced AChR clustering and synaptic differentiation at the muscle midpoint [34–37]. On the other hand, motoneuron-secreted Ach functions as a negative cue to disperse unapposed AChR clusters [38,39]. Taken together, neuronal local clustering activity through agrin and global dispersal activity through Ach precisely shape the subcellular NMJ localization. Muscles in turn secrete the extracellular matrix protein laminin β 2 specifically at AChR-rich “hot spots”, even in the absence of motoneurons [40]. Loss of laminin β 2 leads to declustering of presynaptic calcium channels and other presynaptic components [41].

Inhibitory morphogenetic gradients eliminate synapses from inappropriate locations

Many secreted molecules have been implicated as pro-synaptogenic signals [42,43]. However, recent studies now suggest that they can also act as anti-synaptogenic cues. Furthermore, they can specify subcellular synapse targeting within a neuron.

In *C.elegans*, the motoneuron DA9 forms *en-passant* synapses only in a subdomain of the axon excluding synapses from the most proximal part of the axon. A gradient of two Wnts, LIN-44 and EGL-20, is secreted by hypodermal cells in the tail and prevents premature synapse assembly along the proximal segment of the axon. Loss of Wnts leads to ectopic synapse assembly in the proximal axon, while ectopic Wnt expression inhibits synapse formation in the adjacent axon, where Wnt concentration is highest [44].

Intriguingly, a second morphogenetic gradient formed by Netrin/UNC-6 excludes synapses from the ventral axon and dendrite. In the absence of Netrin/UNC-6 or its receptor UNC-5 synapses assemble in the ventral dendrite [45]. Taken together, two differentially localized morphogenetic gradients restrict synapse assembly to a specific domain within the axon of DA9 neuron.

Wiring specificity controlled at the level of transcription

The growing diversity of molecular players facilitating synaptic specificity poses the question of how their expression is regulated during neural circuit formation. Emerging evidence now implicates several modes of action for transcriptional regulators in synaptic specificity. First, cell-specific expression of transcription factors might contribute to wiring specificity. Secondly, ubiquitous transcription factors might be expressed in certain neurons only at specific developmental time points thereby providing specificity.

At *Drosophila* NMJ, transcription factor Tey regulates synaptic specificity by repressing the expression of repulsive cell surface molecule Toll [46] (Figure 1a). Tey is specifically expressed in muscle M12 but not M13 resulting in the down-regulation of Toll expression in M12 but not M13. Loss of Tey leads to upregulation of Toll on M12 and concomitant inhibition of synapse formation. Conversely misexpression of Tey in M13 causes ectopic innervation.

In the fly visual system, photoreceptor neurons are targeted to different regions in the optic lobe, such as R7 and R8 neurons projecting to the M6 and M3 medulla layers, respectively. Appropriate synaptic layer targeting depends on three transcription factors, differentially expressed in R7 and R8 [47]. R8 neurons express Senseless, which in turn regulates the expression of R8 specific cell surface molecule Capricious [5]. R7 neurons, on the other hand, express the transcription factors Prospero and NF-YC. Interestingly, NF-YC is essential for active repression of R8 targeting program. In NF-YC mutants, R7 axons terminate in M3 due to misexpression of senseless and Capricious.

Synaptic layer specificity of R7 and R8 photoreceptor neurons is also regulated in a temporal manner. Petrovic and coworkers showed in a recent study that the temporal expression of Sequoia transcription factor is a major determinant for correct synaptic layer targeting [48]. The Sequoia-mediated axon targeting is mediated through the ubiquitously expressed cell adhesion molecule CadherinN. Transient expression of sequoia in R7 and R8 induces distinct narrow time windows, in which R7 and R8 cells are competent for synapse formation mediated by CadherinN.

Conclusions

Correct wiring of neural circuits is a prerequisite for proper function of the nervous system. Over the last decade we have learned that nature evolved many different strategies to convey synaptic specificity not only at the cellular but also at the subcellular level. Furthermore, spatial and temporal transcriptional regulation of these cues adds another layer of complexity to wiring specificity. None of the mechanisms described above are mutually

exclusive; rather multiple, redundant processes might contribute to the many ways of synapse specificity.

So far, many extrinsic cues as well as cell surface molecules have been implicated in setting up precise connectivity. In the future, much effort needs to be focused on how these ligands and receptors employ downstream pathways, which eventually execute precise synapse assembly.

Finally, some wiring specificity molecules have been previously identified in other developmental processes such as polarity establishment and axon guidance. Moreover, they can often act both as stimulatory and inhibitory cues depending on the cellular context. Whether these molecules employ the same or different downstream signaling pathways remains an open question.

References and recommended reading

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

• of special interest

•• of outstanding interest

1. Yamagata M, Sanes JR. Dscam and Sidekick proteins direct lamina-specific synaptic connections in vertebrate retina. *Nature*. 2008; 451:465–469. [PubMed: 18216854] Through knock-down and ectopic expression experiments the authors show that homophilic interaction of immunoglobulin-superfamily (IgSF) proteins Sidekicks and Dscams is necessary and sufficient to target synaptic partners to the same retinal sublamina within the IPL.
2. Yamagata M, Weiner JA, Sanes JR. Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. *Cell*. 2002; 110:649–660. [PubMed: 12230981]
3. Kohsaka H, Nose A. Target recognition at the tips of postsynaptic filopodia: accumulation and function of Capricious. *Development*. 2009; 136:1127–1135. [PubMed: 19270171]
4. Kurusu M, Cording A, Taniguchi M, Menon K, Suzuki E, Zinn K. A screen of cell-surface molecules identifies leucine-rich repeat proteins as key mediators of synaptic target selection. *Neuron*. 2008; 59:972–985. [PubMed: 18817735]
5. Shinza-Kameda M, Takasu E, Sakurai K, Hayashi S, Nose A. Regulation of layer-specific targeting by reciprocal expression of a cell adhesion molecule, capricious. *Neuron*. 2006; 49:205–213. [PubMed: 16423695]
6. Shishido E, Takeichi M, Nose A. Drosophila synapse formation: regulation by transmembrane protein with Leu-rich repeats, CAPRICIOUS. *Science*. 1998; 280:2118–2121. [PubMed: 9641918]
7. Hong W, Zhu H, Potter CJ, Barsh G, Kurusu M, Zinn K, Luo L. Leucine-rich repeat transmembrane proteins instruct discrete dendrite targeting in an olfactory map. *Nat Neurosci*. 2009; 12:1542–1550. [PubMed: 19915565]
8. Craig AM, Kang Y. Neurexin-neuroligin signaling in synapse development. *Curr Opin Neurobiol*. 2007; 17:43–52. [PubMed: 17275284]
9. Klein R. Bidirectional modulation of synaptic functions by Eph/ephrin signaling. *Nat Neurosci*. 2009; 12:15–20. [PubMed: 19029886]
10. Biederer T, Sara Y, Mozhayeva M, Atasoy D, Liu X, Kavalali ET, Sudhof TC. SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science*. 2002; 297:1525–1531. [PubMed: 12202822]
11. Pecho-Vrieseling E, Sigrist M, Yoshida Y, Jessell TM, Arber S. Specificity of sensory-motor connections encoded by Sema3e-PlxnD1 recognition. *Nature*. 2009; 459:842–846. [PubMed: 19421194] In this study the authors identified Semaphorin3A and its receptor PlexinD1 as a crucial inhibitory recognition system for specific sensory-motor reflex arc assembly. Changing the

expression of Sema3A or PlxnD1 in sensory and motor neurons leads to rewiring of specific monosynaptic circuits.

12. Inaki M, Yoshikawa S, Thomas JB, Aburatani H, Nose A. Wnt4 is a local repulsive cue that determines synaptic target specificity. *Curr Biol.* 2007; 17:1574–1579. [PubMed: 17764943] In this study the authors describe Wnt4 as inhibitory signal that prevents ectopic synapse formation at *Drosophila* neuromuscular junction. Wnt4 is expressed by M13 but not M12 muscle. In *wnt4* mutant animals, neurons, which normally innervate M12, start to form aberrant connections with M13 muscle.
13. Rose D, Zhu X, Kose H, Hoang B, Cho J, Chiba A. Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in *Drosophila*. *Development.* 1997; 124:1561–1571. [PubMed: 9108372]
14. Flanagan JG. Neural map specification by gradients. *Curr Opin Neurobiol.* 2006; 16:59–66. [PubMed: 16417998]
15. Galimberti I, Bednarek E, Donato F, Caroni P. EphA4 signaling in juveniles establishes topographic specificity of structural plasticity in the hippocampus. *Neuron.* 65:627–642. [PubMed: 20223199] This work precisely describes the topographic distribution of large terminal arborizations of mossy fibers in CA3. Furthermore the authors show that EphA4 signaling and its graded expression is required for accurate presynaptic topographic map.
16. Komiyama T, Luo L. Development of wiring specificity in the olfactory system. *Curr Opin Neurobiol.* 2006; 16:67–73. [PubMed: 16377177]
17. Jefferis GS, Vyas RM, Berdnik D, Ramaekers A, Stocker RF, Tanaka NK, Ito K, Luo L. Developmental origin of wiring specificity in the olfactory system of *Drosophila*. *Development.* 2004; 131:117–130. [PubMed: 14645123]
18. Komiyama T, Sweeney LB, Schuldiner O, Garcia KC, Luo L. Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. *Cell.* 2007; 128:399–410. [PubMed: 17254975]
19. Chao DL, Ma L, Shen K. Transient cell-cell interactions in neural circuit formation. *Nat Rev Neurosci.* 2009; 10:262–271. [PubMed: 19300445]
20. Ango F, Wu C, Van der Want JJ, Wu P, Schachner M, Huang ZJ. Bergmann glia and the recognition molecule CHL1 organize GABAergic axons and direct innervation of Purkinje cell dendrites. *PLoS Biol.* 2008; 6:e103. [PubMed: 18447583]
21. Colon-Ramos DA, Margeta MA, Shen K. Glia promote local synaptogenesis through UNC-6 (netrin) signaling in *C. elegans*. *Science.* 2007; 318:103–106. [PubMed: 17916735]
22. Shen K, Bargmann CI. The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. *Cell.* 2003; 112:619–630. [PubMed: 12628183]
23. 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. [PubMed: 15035988]
24. Liu XB, Low LK, Jones EG, Cheng HJ. Stereotyped axon pruning via plexin signaling is associated with synaptic complex elimination in the hippocampus. *J Neurosci.* 2005; 25:9124–9134. [PubMed: 16207871]
25. Hooks BM, Chen C. Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse. *Neuron.* 2006; 52:281–291. [PubMed: 17046691]
26. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, et al. The classical complement cascade mediates CNS synapse elimination. *Cell.* 2007; 131:1164–1178. [PubMed: 18083105] Through expression profiling of retinal ganglion cells the authors identified components of the classical complement cascade necessary for synapse elimination in visual circuit maturation. Mutants in the complement cascade retain excess retinal innervation by lateral geniculate neurons.
27. Ding M, Chao D, Wang G, Shen K. Spatial regulation of an E3 ubiquitin ligase directs selective synapse elimination. *Science.* 2007; 317:947–951. [PubMed: 17626846]
28. Miles R, Toth K, Gulyas AI, Hajos N, Freund TF. Differences between somatic and dendritic inhibition in the hippocampus. *Neuron.* 1996; 16:815–823. [PubMed: 8607999]
29. Pouille F, Scanziani M. Routing of spike series by dynamic circuits in the hippocampus. *Nature.* 2004; 429:717–723. [PubMed: 15170216]

30. Di Cristo G, Wu C, Chattopadhyaya B, Ango F, Knott G, Welker E, Svoboda K, Huang ZJ. Subcellular domain-restricted GABAergic innervation in primary visual cortex in the absence of sensory and thalamic inputs. *Nat Neurosci.* 2004; 7:1184–1186. [PubMed: 15475951]
31. 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. [PubMed: 15479642] In this publication the authors demonstrate that a gradient of the IgSF protein neurofascin targets GABAergic basket cells innervation to the axon initial segment (AIS) of Purkinje cells. Neurofascin expression is highest at AIS and decreases towards the cell soma. Disruption of the neurofascin gradient leads to aberrant axon targeting and decreases synapse formation.
32. Kim N, Burden SJ. MuSK controls where motor axons grow and form synapses. *Nat Neurosci.* 2008; 11:19–27. [PubMed: 18084289]
33. Lin W, Burgess RW, Dominguez B, Pfaff SL, Sanes JR, Lee KF. Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. *Nature.* 2001; 410:1057–1064. [PubMed: 11323662]
34. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, Hubbard SR, Dustin ML, Burden SJ. Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell.* 2008; 135:334–342. [PubMed: 18848351]
35. Nitkin RM, Smith MA, Magill C, Fallon JR, Yao YM, Wallace BG, McMahan UJ. Identification of agrin, a synaptic organizing protein from Torpedo electric organ. *J Cell Biol.* 1987; 105:2471–2478. [PubMed: 2826489]
36. Strohlic L, Cartaud A, Cartaud J. The synaptic muscle-specific kinase (MuSK) complex: new partners, new functions. *Bioessays.* 2005; 27:1129–1135. [PubMed: 16237673]
37. Zhang B, Luo S, Wang Q, Suzuki T, Xiong WC, Mei L. LRP4 serves as a coreceptor of agrin. *Neuron.* 2008; 60:285–297. [PubMed: 18957220]
38. Lin W, Dominguez B, Yang J, Aryal P, Brandon EP, Gage FH, Lee KF. Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-dependent mechanism. *Neuron.* 2005; 46:569–579. [PubMed: 15944126]
39. Misgeld T, Kummer TT, Lichtman JW, Sanes JR. Agrin promotes synaptic differentiation by counteracting an inhibitory effect of neurotransmitter. *Proc Natl Acad Sci U S A.* 2005; 102:11088–11093. [PubMed: 16043708]
40. Patton BL, Miner JH, Chiu AY, Sanes JR. Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. *J Cell Biol.* 1997; 139:1507–1521. [PubMed: 9396756]
41. Nishimune H, Sanes JR, Carlson SS. A synaptic laminin-calcium channel interaction organizes active zones in motor nerve terminals. *Nature.* 2004; 432:580–587. [PubMed: 15577901]
42. Salinas PC, Zou Y. Wnt signaling in neural circuit assembly. *Annu Rev Neurosci.* 2008; 31:339–358. [PubMed: 18558859]
43. Lai KO, Ip NY. Synapse development and plasticity: roles of ephrin/Eph receptor signaling. *Curr Opin Neurobiol.* 2009; 19:275–283. [PubMed: 19497733]
44. Klassen MP, Shen K. Wnt signaling positions neuromuscular connectivity by inhibiting synapse formation in *C. elegans*. *Cell.* 2007; 130:704–716. [PubMed: 17719547]
45. Poon VY, Klassen MP, Shen K. UNC-6/netrin and its receptor UNC-5 locally exclude presynaptic components from dendrites. *Nature.* 2008; 455:669–673. [PubMed: 18776887] In these two studies the authors show that gradients of two secreted molecules, Wnt and netrin, precisely position presynaptic en-passant boutons along the axon of DA9 motor neuron in *C.elegans*. While netrin is present as a ventral-dorsal gradient and inhibits presynapse formation on the ventral dendrite and axon, Wnt is expressed as a posterior-anterior gradient and inhibits presynapse assembly in the proximal dorsal axon.
46. Inaki M, Shinza-Kameda M, Ismat A, Frasnch M, Nose A. Drosophila Tey represses transcription of the repulsive cue Toll and generates neuromuscular target specificity. *Development.* 137:2139–2146.

47. Morey M, Yee SK, Herman T, Nern A, Blanco E, Zipursky SL. Coordinate control of synaptic-layer specificity and rhodopsins in photoreceptor neurons. *Nature*. 2008; 456:795–799. [PubMed: 18978774]
48. Petrovic M, Hummel T. Temporal identity in axonal target layer recognition. *Nature*. 2008; 456:800–803. [PubMed: 18978776] These two studies provide compelling evidence that laminar specificity within the fly optic lobe is under spatial as well as temporal transcriptional control. Spatial transcriptional control is achieved through cell specific expression of transcription factors NY-C and prospero in R7 cells and senseless in R8 cell, which consequently induces the expression of R8-specific targeting molecule Capricious. Temporal specificity is obtained by transient expression of transcription factor sequoia in R7 and R8 and concomitant induction of cell surface protein CadherinN.

Figure 1.

Examples of negative and positive mechanisms mediating synaptic specificity. Positive and negative cues are depicted in red and blue colors respectively. (a) Synaptic specificity at *Drosophila* NMJ. Left: Wnt4 specifically expressed by muscle M13 acts as an inhibitory cue, thereby allowing appropriate synapse formation between muscle M12 and neuron MN12. Right: Transcriptional regulation of synapse specificity: Transcription factor Tey regulates synaptic match-making by repressing the expression of repulsive cell surface molecule Toll in M12 but not M13. (b) Specific Purkinje cell innervation (black cell) by basket cells (green) and stellate neurons (yellow). Upper panel: Bergmann glia (orange), so called guidepost cells, express the cell surface molecule CHL1, which is instructive for

proper guidance of Stellate axons (yellow) towards Purkinje dendrites. Lower panel:
Example of subcellular specificity: Basket cells innervate Purkinje cells specifically at the
AIS due to a neurofascin gradient along the purkinje cell soma and AIS.

Table 1

Overview of synaptic specificity molecules. Red color represents synapse specificity promoting factors, while molecules colored in blue are inhibitory factors.

Mechanism	Protein	Expression	Organism	References
<i>Cell surface molecules:</i>				
IgSF proteins	Sdk1, Sdk2,	pre & post	Chicken	[1,2]
	Dscam, DscamL	pre & post	Chicken	[2]
	SYG-1,	pre	C.elegans	[23,24]
	SYG-2	guidepost cells	C.elegans	[24]
	Neurofascin 186	pre	Mouse	[32]
	CHL1	guidepost cells	Mouse	[21]
LRR proteins	Capricious,	pre & post	Drosophila	[3,5,7,8,9]
	Tartan,	pre	Drosophila	[3]
	Toll	post	Drosophila	[4,47]
Cadherins	CadN	pre	Drosophila	[49]
Receptors	UNC-40/DCC	pre	C.elegans	[22]
	UNC-5	pre	C.elegans	[46]
	LIN-17/Frizzled	pre	C.elegans	[45]
	Lrp4/MuSK	post	Mouse	[35,37,38]
	AchR	post	Mouse	[33,34]
Semaphorins/Plexins	Sema3e	post	Mouse	[13]
	Plxnd1	pre	Mouse	[13]
	Sema1a	post	Drosophila	[19]
	PlxA3	?	Mouse	[25]
<i>Secreted molecules:</i>				
	Wnt4	post	Drosophila	[14]
	Eph4A	surrounding tissue	Mouse	[25]
	UNC-6/Netrin	surrounding tissue / guidepost cells surrounding tissue	C.elegans	[22,46]
	Wnt	surrounding tissue	C.elegans	[45]
	Agrin	pre	Mouse	[36]
	Acetylcholine	pre	Mouse	[40]
	laminin β 2	post	Mouse	[41]
<i>Intracellular molecules</i>				
Transcription factors	Senseless	pre	Drosophila	[48]
	NF-YC	pre	Drosophila	[48]
	Prospero	pre	Drosophila	[48]
	Sequoia	pre	Drosophila	[49]
	Tey	pre	Drosophila	[47]
Other molecules	C1q, C3	pre	Mouse	[27]
	SKR-1	pre	C.elegans	[28]